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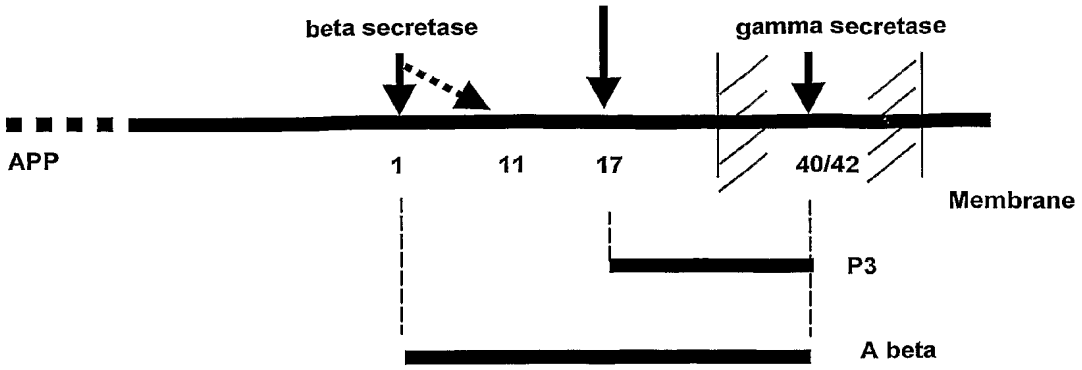
(54) Title: METHOD OF IDENTIFYING A COMPOUND THAT CHANGES THE AMYLOID-BETA PRECURSOR PROTEIN PROCESSING IN A CELL



(57) Abstract: The present invention relates to methods of identifying a compound that changes the amyloid-beta precursor protein processing in a cell. The invention also relates to methods for changing the amyloid-beta precursor protein processing of a cell, and a method for diagnosing a pathological condition involving cognitive impairment or susceptibility to this condition in a subject.

WO 2005/024057 A1

Method of identifying a compound that changes the amyloid-beta precursor protein processing in a cell.

The present invention relates to methods of identifying a compound that changes the amyloid-beta precursor protein processing in a cell. The invention also relates to methods for changing the amyloid-beta precursor protein processing of a cell.

Alzheimer's disease (AD) is a neurological disorder that is clinically characterized by the progressive loss of intellectual capacities: initially memory, and later on by disorientation, impairment of judgment and reasoning, commonly referred to as cognitive impairment, and ultimately full dementia. The patients finally fall into a severely debilitated, immobile state between 4 and 12 years after onset of the disease. Worldwide, about 20 million people suffer from Alzheimer's disease. The pathological hallmarks of AD are the presence of extracellular amyloid plaques and intracellular tau tangles in the brain, which are associated with neuronal degeneration (Ritchie and Lovestone (2002)).

A small fraction of AD cases are caused by autosomal dominant mutations in the genes encoding presenilin 1 and 2 (PS1; PS2) and the amyloid-beta precursor protein (APP). It has been shown that mutations in APP, PS1 and PS2 alter amyloid-beta precursor protein metabolism such that more of the insoluble, pathogenic amyloid beta 1-42 is produced in the brain. Following secretion, these amyloid beta 1-42 peptides form amyloid fibrils more readily than the amyloid beta 1-40 peptides, which are predominantly produced in healthy people. These insoluble, amyloid fibrils are then deposited in the amyloid plaques.

The amyloid beta peptides are generated from the membrane anchored APP, after cleavage by beta secretase and

gamma secretase at position 1 and 42, respectively (Figure 1)(Annaert and De Strooper (2002)). The gamma secretase can also cleave at position 40. In addition, high activity of beta secretase results in a shift of the cleavage at position 1 to position 11. Cleavage of amyloid-beta precursor protein by alpha secretase activity and gamma secretase activity at position 17 and 40 or 42 generates the non-pathological p3 peptide. Beta secretase was identified as the membrane anchored aspartyl protease BACE, while gamma secretase is a protein complex comprising presenilin 1 (PS1) or presenilin 2 (PS2), nicastrin, Anterior Pharynx Defective 1 (APH1) and Presenilin Enhancer 2 (PEN2). Of these proteins, the presenilins are widely thought to constitute the catalytic activity of the gamma secretase, while the other components play a role in the maturation and localization of the complex. The identity of the alpha secretase is still illustrious, although some results point towards the proteases ADAM 10 and TACE, which could have redundant functions.

It has been shown that injection of amyloid beta fibrils in the brains of P301L tau transgenic mice enhances the formation of neurofibrillary tangles, placing the amyloid beta peptide on top of the neurotoxic cascade (Gotz et al. (2001)). Although no mutations in PS1, PS2 and amyloid-beta precursor protein have been identified in late onset AD patients, the pathological hallmarks are highly similar to the early onset AD patients. Therefore, it is generally accepted that aberrant increased amyloid peptide levels in the brains of late onset AD patients are also the cause of the disease. These increased levels of amyloid beta peptide could originate progressively with age from disturbed amyloid-beta precursor protein processing (e.g. high cholesterol levels enhance amyloid beta peptide production) or from decreased catabolism of the peptide.

Since the socio-economical impact of AD is large, the need for an effective therapy is urgent. Because the cholinergic neurons are the first neurons to degenerate during AD, levels of the neurotransmitter acetylcholine decrease, resulting in the progressive loss of memory. Therefore, the major current AD therapies are focused on the inhibition of the acetylcholinesterase enzyme, leading to an increased concentration of the acetylcholine. However, this therapy does not halt the progression of the disease.

Therapies aimed at decreasing the levels of amyloid beta peptides in the brain, are heavily investigated and will become very important. Most of these therapies are focused on the perturbed amyloid-beta precursor protein processing and target directly beta- or gamma secretase activity. However, targeting these proteins has not yielded any new drugs yet, because of the difficulty to find specific drugs and the suspected serious side effects.

Therefore the identification of alternative drug targets within the amyloid-beta precursor protein processing pathway is of great interest, since this would allow a direct interference with the production of the pathological amyloid beta 1-42 peptide, which should block the neurotoxic cascade induced by the latter.

The present invention relates to a method of identifying a compound that changes the amyloid-beta precursor protein processing in a cell, comprising:

(a) providing a host cell expressing a polypeptide having a amino acid sequence selected from the group consisting of SEQ ID NO: 15-28, or a fragment, or a derivative thereof;

(b) determining a first activity level of the polypeptide by measuring the level of one or more second messengers of the polypeptide;

(c) exposing the host cell to a compound;

(d) determining a second activity level of the polypeptide by measuring the level of the second messengers after exposing of the host cell to the compound; and

(e) identifying the compound by which the second activity level is less than the first activity level.

The polypeptides of this invention, when overexpressed or activated, induce the level of secreted amyloid beta 1-42, amyloid beta 1-40, and amyloid beta 1-x, where x ranges from 19-42.

Specifically, the amyloid beta peptides 1-42, 1-40, 1-39, 1-38, 1-37 are often seen in the cerebral spinal fluid. The level of these amyloid beta peptides in Alzheimer patients is increased compared to the levels of these peptides in healthy persons. The amyloid beta peptides 1-42, 1-40, 1-39, 1-38, 1-37 can be found in plaques. Thus, reducing the levels of these amyloid beta peptide is beneficial for patients with cognitive impairment. Therapeutically relevant drug targets may yield an increase in amyloid beta 1-42 levels. The pharmacological inhibition of these targets results in a decrease of amyloid beta levels.

The polypeptides of this invention are G-protein coupled receptors (GPCRs) and can be inhibited by small molecules. All GPCRs share a common architecture of 7 transmembrane domains, an extracellular N-terminus and an intracellular C-terminus. The major signal transduction cascades activated by GPCRs are initiated by the activation of heterotrimeric G-proteins (Wess (1998)), built from three different proteins; the G_α, G_β and G_γ subunits. The signal transduction cascade starts with the activation of the receptor by an agonist. Transformational changes in the receptor are then translated down to the G-protein. The G-protein dissociates into the G_α subunit and the $G_{\beta\gamma}$ subunit. Both subunits dissociate from the receptor and are both capable of initiating different

cellular responses. Best known are the cellular effects that are initiated by the G_α subunit. It is for this reason that G-proteins are categorized by their G_α subunit. The G-proteins are divided into four groups: G_s , $G_{i/o}$, G_q and $G_{12/13}$. Each of these G-proteins is capable of activating an effector protein, which results in changes in second messenger levels in the cell. The changes in second messenger level are the triggers that make the cell respond to the extracellular signal in a specific manner. The activity of a GPCR can be measured by measuring the activity level of the second messenger.

The two most important second messengers in the cell are cAMP and Ca^{2+}. The α-subunit of the G_s class of G-proteins is able to activate adenylyl cyclase, resulting in an increased turnover from ATP to cAMP. The α-subunit of $G_{i/o}$ G-proteins does exactly the opposite and inhibits adenylyl cyclase activity resulting in a decrease of cellular cAMP levels. Together, these two classes of G-proteins regulate the second messenger cAMP. Ca^{2+} is regulated by the α-subunit of the G_q class of G-proteins. Through the activation of phospholipase C phosphatidylinositol 4,5-bisphosphate (PIP2) from the cell membrane are hydrolyzed to inositol 1,4,5-trisphosphate and 1,2-diacylglycerol, both these molecules act as second messengers. Inositol 1,4,5-trisphosphate binds specific receptors in the endoplasmatic reticulum, resulting in the opening of Ca^{2+} channels and release of Ca^{2+} in the cytoplasm.

Second messenger activation can be measured by several different techniques, either directly by ELISA or radioactive technologies or indirectly by reporter gene analysis.

A host cell expressing a polypeptide of the present invention can be a cell with endogenous expression of the polypeptide or a cell overexpressing the polypeptide e.g. by transduction. When the endogenous expression of the polypeptide of the present invention is not sufficient for a

first activity level of the second measure that can easily be measured, overexpression of the polypeptide can be applied. Overexpression has the advantage that the first activity level of the second messenger is higher than the activity level by endogenous expression.

Preferably the method according to the present invention further comprises contacting the host cell with an agonist for the polypeptide before determining the first activity level. The addition of an agonist further stimulates the polypeptides of the present invention, thereby further increasing the activity level of the second messenger.

Another embodiment relates to the method according to the present invention further comprising

(f) contacting a population of mammalian cells expressing a polypeptide having a amino acid sequences selected from the group consisting of SEQ ID NO: 15-28, or a fragment, or a derivative thereof, with the compound identified in step (e); and

(g) identifying the compound that changes the amyloid-beta precursor protein processing in the cells.

Amyloid-beta precursor protein is processed into several different amyloid beta peptides species. According to the invention, mompounds are identified that change the APP processing and reduce the level of secreted pathological amyloid beta peptides. Levels of amyloid beta peptides can be measured with specific ELISA's using antibodies specifically recognizing the different amyloid beta peptide species (see e.g. Example 1). Levels of amyloid beta peptides can also be measured by Mass spectrometry analysis (see e.g. Example 7).

According to a particular embodiment of the present invention the polypeptide is FPRL1, as defined by SEQ ID NO: 15.

According to another embodiment of the present invention, the polypeptide is GCGR, as defined by SEQ ID NO:

22. Overexpression of FPRL1 or GCGR (example 1) and/or activation of these receptors (example 4) results in increased levels of amyloid beta peptide 1-42, 1-40 and 1-X, where x ranges from 19-42, compared to negative control levels.

Acoording to a further preferred embodiment of the method according to the present invention, the activity level is determined with a reporter controlled by a promoter, which is responsive to the second messenger.

The reporter is a reporter gene under the regulation of a promoter that responds to the cellular level of second messengers. The reporter gene has a gene product that is easily detected. Reporter genes can be easily transferred to host cells by persons of ordinary skill in the art. The reporter gene can be stably infected in the host cell. The reporter gene may be selected from the group comprising: alkaline phosphatase, enhanced green fluorescent protein, destabilized green fluorescent protein, luciferase or β-galactosidase.

Preferably the promoter is a cyclic AMP-responsive promoter, a NF-KB responsive promoter, or a NF-AT responsive promoter. The cyclic-AMP responsive promoter is responsive for the cyclic-AMP levels in the cell. The NF-AT responsive promoter is sensitive to cytoplasmic Ca^{2+}-levels in the cell. The NF-KB responsive promoter is sensitive for activated NF-κB levels in the cell.

Preferably the reporter is luciferase or β-galactosidase. Luciferase and β-galactosidase are easily available and have a large dynamic range for measuring. In addition, luciferase and β-galactosidase are less expensive which is favorable especially when performing the method of the present invention in a high throughput format.

The present invention further relates to a method for identifying a compound that changes the amyloid-beta precursor protein processing in a cell, comprising:

(a) contacting one or more compounds with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 15-28, or a derivative, or a fragment thereof,

(b) determining the binding affinity of the compound to the polypeptide,

(c) contacting a population of mammalian cells expressing the polypeptide with the compound that exhibits a binding affinity of at least 10 micromolar, and

(d) identifying the compound that changes the amyloid-beta precursor protein processing in the cells.

In addition, the present invention relates to a method for identifying a compound that changes the amyloid-beta precursor protein processing in cells, comprising:

(a) contacting one or more compounds with a polynucleotide or a vector comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1-14

(b) determining the binding affinity of the compound to the polynucleotide or to the vector,

(c) contacting a population of mammalian cells expressing the polynucleotide sequence with the compound that exhibits a binding affinity of at least 10 micromolar, and

(d) identifying the compound that changes the amyloid-beta precursor protein processing of the cells.

The binding affinity of the compound to the polypeptide or polynucleotide can be measured by methods known in the art, such as using surface plasmon resonance biosensors (Biacore), by saturation binding analysis with a labeled compound (e.g. Scatchard and Lindmo analysis), by differential UV spectrophotometer, fluorescence polarisation assay, Fluorometric Imaging Plate Reader (FLIPR®)

system, Fluorescence resonance energy transfer, and Bioluminescence resonance energy transfer. The binding affinity of compounds can also be expressed in a dissociation constant (Kd) or as IC50 or EC50. The IC50 represents the concentration of a compound that is required for 50% inhibition of binding of another ligand to the polypetide. The EC50 represents the concentration required for obtaining 50% of the maximum effect in any assay that measures receptor function. The dissociation constant, Kd, is a measure of how well a ligand binds to the polypeptide , it is equivalent to the ligand concentration required to saturate exactly half of the binding-sites on the polypeptide. Compounds with a high affinity binding have low Kd, IC50 and EC50 values, i.e. in the range of 100 nM to 1 pM; a moderate to low affinity binding relates to a high Kd, IC50 and EC50 values, i.e. in the micromolar range.

Changing the APP processing according to the present invention relates to the reduction of the level of amyloid beta peptide 1-x, whereby x ranges from 19-42 and/or the increase of the level of amyloid beta peptide y-42, whereby y ranges from 1-24. The changes in amyloid beta peptide levels can be measured by e.g. an ELISA with specific antibodies as explained in example 1 or by mass spectrometry analysis (example 7).

For high-throughput purposes, libraries of compounds can be used such as peptide libraries (e.g. LOPAP™, Sigma Aldrich), lipid libraries (BioMol), synthetic compound libraries (e.g. LOPAC™, Sigma Aldrich) or natural compound libraries (Specs, TimTec).

Preferably the compounds are low molecular weight compounds. Low molecular weight compounds, i.e. with a molecular weight of 500 Dalton or less, are likely to have good absorption and permeation in biological systems and are consequently more likely to be successful drug candidates

than compounds with a molecular weight above 500 Dalton (Lipinski et al. (1997)).

According to another preferred embodiment the compounds are peptides. Many GPCRs have a peptide as an antagonist. Peptides can be excellent drug candidates and there are multiple examples of commercially valuable peptides such as fertility hormones and platelet aggregation inhibitors.

According to another preferred embodiment the compounds are natural compounds. Natural compounds are compounds that have been extracted from e.g. plants or compounds that are synthesized on the basis of a natural occurring molecule. Using natural compounds in screens has the advantage that more diverse molecules are screened. Natural compounds have an enormous variety of different molecules. Synthetic compounds do not exhibit such variety of different molecules.

According to another preferred embodiment the compounds are lipids. GPCRs listed in table 1 can have lipids as antagonists. Using lipids as candidate compounds can increase the chance of finding a specific agonist for the polypeptides of the present invention.

Another aspect of the invention relates to a method for changing the amyloid-beta precursor protein processing of a cell, comprising inhibiting the biological activity of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 15-28 and fragments, or derivatives thereof by contacting the cell with an expression inhibitory agent that inhibits the translation in the cell of a polyribonucleotide encoding the polypeptide.

Polypeptides of the present invention increase the level of pathological amyloid beta peptides. Inhibiting the activity of the polypeptide decreases the level of pathological amyloid beta peptides.

According to another preferred embodiment of the present invention the expression inhibitory agent is selected from

the group consisting of an antisense RNA, a ribozyme that cleaves the polyribonucleotide, an antisense oligodeoxynucleotide (ODN), a small interfering RNA (siRNA) that is sufficiently homologous to a portion of the polyribonucleotide such that the siRNA is capable of inhibiting the polyribonucleotide that would otherwise cause the production of the polypeptide, and an antibody reactive to the polypeptide.

According to another advantageous embodiment of the present invention the expression inhibitory agent is a nucleic acid expressing the antisense RNA, a ribozyme that cleaves the polyribonucleotide, an antisense oligodeoxynucleotide (ODN), a siRNA that is sufficiently homologous to a portion of a the polyribonucleotide such that the siRNA is capable of inhibiting the polyribonucleotide that would otherwise cause the production of the polypeptide, or an antibody reactive to the polypeptide

One type of expression-inhibitory agent concerns a nucleic acid that is antisense to a nucleic acid comprising SEQ ID NO: 1-14. For example, an antisense nucleic acid (e.g. DNA) may be introduced into cells in vitro, or administered to a subject in vivo, as gene therapy to inhibit cellular expression of nucleic acids comprising SEQ ID NO: 1-14. Antisense oligonucleotides preferably comprise a sequence containing from about 17 to about 100 nucleotides and more preferably the antisense oligonucleotides comprise from about 18 to about 30 nucleotides. Antisense nucleic acids may be prepared by expression of all or part of a sequence selected from the group consisting of SEQ ID NO: 1-14, in the opposite orientation. Antisense oligonucleotides may also contain a variety of modifications that confer resistance to nucleolytic degradation such as, for example, modified internucleoside linkages, modified nucleic acid bases and/or modified sugars and the like. The antisense oligonucleotides

may also be modified by chemically linking the oligonucleotide to one or more moieties or conjugates to enhance the activity, cellular distribution, or cellular uptake of the antisense oligonucleotide. Such moieties or conjugates include lipids such as cholesterol, cholic acid, thioether, aliphatic chains, phospholipids, polyamines, polyethylene glycol (PEG), or palmityl moieties.

Another type of expression-inhibitory agent is a nucleic acid that is able to catalyze cleavage of RNA molecules. The expression "ribozymes" relates to catalytic RNA molecules capable of cleaving other RNA molecules at phosphodiester bonds in a manner specific to the sequence. The hydrolysis of the target sequence to be cleaved is initiated by the formation of a catalytically active complex consisting of ribozyme and substrate RNA. All ribozymes capable of cleaving phosphodiester bonds in trans, that is to say intramolecularly, are suitable for the purposes of the invention. Apart from ribonuclease P the known naturally occurring ribozymes (hammerhead ribozyme, hairpin ribozyme, hepatitis delta virus ribozyme, Neurospora mitochondrial VS ribozyme, group I and group II introns) are catalysts, which cleave or splice themselves and which act in cis (intramolecularly).

Yet another method of expression-inhibition is by small interfering RNAs (siRNAs). siRNAs mediate the post-transcriptional process of gene silencing by double stranded RNA (dsRNA) that is homologous in sequence to the silenced RNA.

Preferably the nucleotide expressing the expression inhibitory agent is included within a vector. Even more preferred, the vector is an adenoviral, retroviral, adeno-associated viral, lentiviral or a sendaiviral vector.

According to a further preferred embodiment of the present invention, the siRNA comprises a sense strand of 17-

23 nucleotides homologous to a 17-23 nucleotide long nucleotide sequence selected from the group consisting of SEQ ID NO: 1-14 and an antisense strand of 17-23 nucleotides complementary to the sense strand. All nucleotides in the sense and antisense strand base pair, or alternatively there may be mismatches between the sense and antisense strand.

Preferably the siRNA further comprises a loop region connecting the sense and the antisense strand.

A self-complementing single-stranded siRNA molecule according to the present invention comprises a sense portion and an antisense portion connected by a loop region. Preferably, the loop region is 4-30 nucleotides long, more preferably 5-15 nucleotides long and most preferably 8 nucleotides long. In a most preferred embodiment the loop region consists of the sequence UUGCUAUA (SEQ ID NO: 339).

Self-complementary single stranded siRNAs form hairpin loops and are more stable than ordinary dsRNA. In addition, they are more easily produced from vectors.

According to a further advantageous embodiment the expression inhibitory agent is an antisense RNA, ribozyme, antisense oligodeoxynucleotide, or siRNA comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

The nucleotide sequences are selected according to siRNA designing rules that give an improved reduction of the target sequences compared to nucleotide sequences that do not comply with these siRNA designing rules (See PCT/EP03/04362).

A further aspect of the invention relates to a polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

Another aspect of the present invention concerns a polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for use as a medicament.

Yet another aspect of the present invention relates to the use of a polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for the manufacture of a medicament for the treatment of a disease involving cognitive impairment.

Polynucleotides, selected from the group consisting of SEQ ID NO: 29-338, can be used in expression inhibitory agents inhibiting the expression of polypeptides of the present invention as described above.

Preferably the polynucleotide is a siRNA.

According to another aspect, the invention relates to a vector comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

According to yet another aspect, the present invention relates to a vector comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for use as a medicament.

Furthermore, the present invention relates to the use of a vector comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for the manufacture of a medicament for the treatment of a disease involving cognitive impairment.

Preferably, the vector encodes a siRNA, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

Preferably the vector is an adenoviral, retroviral, adeno-associated viral, lentiviral or a sendaiviral vector.

In a preferred embodiment of the present invention the disease is Alzheimer's disease.

According to another aspect the present invention concerns a method for diagnosing a pathological condition involving cognitive impairment or a susceptibility to the condition in a subject comprising:

(a) obtaining a sample of the subject's mRNA corresponding to a nucleic acid selected from the group consisting of SEQ ID NO: 1-14, or a sample of the subject's genomic DNA corresponding to a genomic sequence of a nucleic acid selected from the group consisting of SEQ ID NO: 1-14

(b) determining the nucleic acid sequence of the subject's mRNA or genomic DNA;

(c) comparing the nucleic acid sequence of the subject's mRNA or genomic DNA with a nucleic acid selected from the group consisting of SEQ ID NO: 1-14 or with a genomic sequence encoding a nucleic acid selected from the group consisting of SEQ ID NO: 1-14 obtained from a database; and

(d) identifying any difference(s) between the nucleic acid sequence of the subject's mRNA or genomic DNA and the nucleic acid selected from the group consisting of SEQ ID NO 1-14 or the genomic sequence encoding a nucleic acid selected from the group consisting of SEQ ID NO: 1-14 obtained from a database.

It is well understood in the art that databases such as GenBank, can be searched to identify genomic sequences that contain regions of identity (exons) to a nucleic acid. Such genomic sequences encode for the nucleic acid.

According to a further aspect, the present invention relates to method for diagnosing a pathological condition involving cognitive impairment or a susceptibility to the condition in a subject, comprising determining the amount of polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 15-28 in a biological sample, and comparing the amount with the amount of the polypeptide in healthy subjects, wherein an increase of the amount of polypeptide compared to the healthy subjects is indicative of the presence of the pathological condition.

Preferably the pathological condition is Alzheimer's disease.

The term "amyloid beta peptide species" refers to amyloid beta peptides with different composition that are processed from the amyloid beta precursor protein (APP). Examples of the species are 1-40, 1-42, y-42, whereby y ranges from 1-24, and 1-x whereby x ranges from 19-42.

The term "expression" comprises both endogenous expression and overexpression by transduction.

The term "compound" comprises organic and inorganic compounds, such as synthetic molecules, peptides, lipids, and natural compounds.

The term "agonist" refers to a ligand that activates the receptor the ligand binds to.

The term "polypeptide" relates to a protein, fractions of a protein, peptides, oligopeptides, or enzymes.

The term "derivatives of a polypeptide" relate to those peptides, oligopeptides, polypeptides, proteins and enzymes that comprise at least about 10 contiguous amino acid residues of the polypeptide and that retain the biological activity of the protein, e.g. polypeptides that have amino acid mutations compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may further comprise additional naturally-occurring, altered, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the polypeptide. It may also contain one or more non-amino acid substituents compared to the amino acid sequence of a naturally-occurring form of the polypeptide, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence.

The term "fragment of a polypeptide" relates to peptides, oligopeptides, polypeptides, proteins and enzymes that comprise at least about 5 contiguous amino acid

residues, preferably at least 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 contiguous amino acid residues, and exhibit substantially a similar, but not necessarily identical, activity as the complete sequence.

The term "polynucleotide" refers to all nucleic acids, such as DNA and RNA, oligonucleotides. It also includes nucleic acids with modified backbones such as peptide nucleic acid, polysiloxane, and 2'-O-(2-methoxy)ethylphosphorothioate.

The term "derivatives of a polynucleotide" relates to DNA- and RNA- molecules, and oligonucleotides that comprise at least about 10 contiguous nucleic acid residues of the polynucleotide, e.g. polynucleotides that have nucleic acid mutations compared to the nucleic acid sequence of a naturally-occurring form of the polynucleotide. A derivative may further comprise nucleic acids with modified backbones such as peptide nucleic acid (PNA), polysiloxane, and 2'-O-(2-methoxy)ethyl-phosphorothioate, non-naturally occurring nucleic acid residues, or one or more nuclei acid substituents, such as methyl-, thio-, sulphate, benzoyl-, phenyl-, amino-, propyl-, chloro-, and methanocarbanucleosides, or a reporter molecule to facilitate its detection.

The term "fragment of a polynucleotide" relates to oligonucleotides that comprise at least about 5 contiguous nucleic acid residues, preferably at least 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 contiguous nucleic acid residues, and exhibit substantially a similar, but not necessarily identical, activity as the complete sequence.

Figure legends:

Figure 1: APP processing: The membrane anchored amyloid precursor protein (APP) is processed by two pathways: the amyloidogenic and non amyloidogenic pathway. In the latter pathway, APP is cleaved first by alpha secretase and then by gamma secretase, yielding the p3 peptides (17-40 or 17-42). The amyloidogenic pathway generates the pathogenic amyloid beta peptides (A beta) after cleavage by beta- and gamma-secretase respectively. The numbers depicted are the positions of the amino acids comprising the A beta sequences.

Figure 2: Evaluation of the APP processing assay: Positive (PS1G384L; PS1L392V and BACE1) and negative (eGFP, LacZ and empty) control viruses are infected in Hek293APPwt at random MOI, mimicking a screening. A and B: Transduction is performed respectively with 1 and 0.2 µl of virus and amyloid beta 1-42 levels are performed. Data are represented as relative light units and correlate to pM of amyloid beta 1-42.

Figure 3: Screening results: Hek293 APPwt cells are transduced with 0.2 µl of a collection of Ad5/GPCRs and amyloid beta 1-42 levels are monitored through ELISA. The data points from the plate comprising several Ad5/FPRL1 and Ad5/GCGR viruses are depicted. Viruses scoring above the cut-off value, which is calculated based on the formula [average + (3* standard deviation)], are considered as positives and thus stimulate amyloid beta production (1-42, 1-40). Data are represented as relative light units and correlate to pM of amyloid beta 1-42.

Figure 4: Confirmation of the involvement of FPRL1 and GCGR: Hek293 APPwt cells are transduced with Ad5/FPRL1, Ad5/GCGR and with 4 negative control viruses (Ad5/empty, Ad5/LacZ, Ad5/eGFP and Ad5/luciferase) at different MOIs (2-1250). Resulting amyloid beta 1-42, 1-40 and 1-x peptides were measured with the appropriate ELISA's. Data are

represented in pM or as relative light units (rlu), which correlates to pM of amyloid beta 1-x.

Figure 5: reporter gene analysis

A: Glucagon dose response curve on HEK293 cells expressing the human glucagon receptor. Hek 293 cells are transduced with an adenovirus harboring the luciferase gene under the control of a cAMP dependent promoter and a virus harboring the glucagon receptor cDNA. 40 h after infection the cells were treated with increasing amounts of glucagon. Cells were lysed and the luciferase activity determined. Glucagon has a pEC_{50} value of 6.6. EC_{50} values were calculated with GraphPad Prism using non-linear regression.

B Glucagon dose response curve on HEK293 cells expressing the human glucagon receptor. Hek 293 cells are transduced with an adenovirus harboring the luciferase gene under the control of a Ca^{2+} dependent promoter (NFAT elements) and a virus harboring the glucagon receptor cDNA. 40 h after infection the cells were treated with increasing amounts of glucagon. Cells were lysed and the luciferase activity determined. Glucagon has a pEC_{50} value of 6.6. EC_{50} values were calculated with GraphPad Prism using non-linear regression.

C: fMLF dose responds curve on HEK293 cells expressing the human FPRL1 receptor. Hek 293 cells are transduced with an adenovirus harboring the luciferase gene under the control of a cAMP dependent promoter (CRE elements) and a virus harboring the FPRL1 receptor cDNA. 40 h after infection the cells were treated with increasing amounts of fMLF and 10 μM forskolin. Cells were lysed and the luciferase activity determined. fMLF has a pEC_{50} value of 7.4. EC_{50} values were calculated with GraphPad Prism using non-linear regression.

Figure 6: effect of agonists: Hek293 APPwt cells are transduced with Ad5/GCGR (A), Ad5/FPRL1 (B) and Ad5/empty (A and B), at a MOI of 50. After 24h, the viruses are removed

and medium containing respectively 5nM glucagon (A), 1mM fMLF (B) or vehicle only is added. 24h later, the conditioned medium is collected and resulting amyloid beta 1-42 peptides were measured with the amyloid beta 1-42 ELISA. Data are represented as pM.

Figure.7: ClustalW protein sequence alignment of GCGR with its closest relatives, being GLP1R and GLP2R. A second ClustalW alignment of the glucagon and the glucagon like peptides is shown. In addition, in table 6 the percentage of identity and similarity of other close homologues is shown.

Figure 8: ClustalW protein sequence alignment of FPRL1 with its closest relatives, being FPR1 and FPRL2. In addition, in table 5 the percentage of identity and similarity of other close homologues is shown.

TABLE 1: GPCRs involved in APP processing:

Accession	Code	Description	SEQ ID NO:	
			DNA	Protein
NM_001462	FPRL1	formyl peptide receptor-like 1	1	15
NM_002030	FPRL2	formyl peptide receptor-like 2	2	16
NM_002029	FPR1	formyl peptide receptor 1	3	17
NM_001506	GPR32	G protein-coupled receptor 32	4	18
NM_004072	CMKLR1	chemokine-like receptor 1	5	19
NM_001736	C5R1	complement component 5 receptor 1 (C5a ligand)	6	20
NM_004778	GPR44	G protein-coupled receptor 44	7	21
NM_000160	GCGR	glucagon receptor	8	22
NM_002062	GLP1R	glucagon-like peptide 1 receptor	9	23
NM_004246	GLP2R	glucagon-like peptide 2 receptor	10	24
NM_000164	GIPR	gastric inhibitory polypeptide receptor	11	25
NM_004624	VIPR1	vasoactive intestinal peptide receptor 1	12	26
NM_002980	SCTR	secretin receptor	13	27
NM_003382	VIPR2	vasoactive intestinal peptide receptor 2	14	28

TABLE2: buffers and solutions used for ELISA

Buffer 42	30mM $NaHCO_3$, 70mM Na_2CO_3, 0.05% NaN_3, pH9.6
Casein buffer	0.1% casein in PBS 1x
EC Buffer	20mM sodium phosphate, 2mM EDTA, 400mM NaCl, 0.2% BSA, 0.05% CHAPS, 0.4% casein, 0.05% NaN_3, pH7
Buffer C	20mM sodium phosphate, 2mM EDTA, 400mM NaCl, 1% BSA, pH7
PBS 10x	80g NaCl + 2g KCl + 11.5g $Na_2HPO_4.7H_2O$ + 2g KH_2PO_4 in 1 l milli Q, pH 7.4
PBST	PBS 1x with 0.05% Tween 20

TABLE 3: Primers used in the quantitative real time PCR analysis for GPCR expression levels

Gene	Primer name	SEQ ID NO:	Primer sequence
FPRL1	FPRL1_Hs_For	340	CCACAAAAAGGGCATGATTAAATC
	FPRL1_Hs_Rev	341	TGAAAGGGAAACCAACAGATGA

TABLE 4: Ct values obtained during quantitative real time PCR: Total human brain, human cerebral cortex or human hippocampus RNA is tested for the presence of FPRL1 RNA via quantitative real time PCR. GAPDH RNA is used to normalize all samples (□Ct).

Tissue	Ct		ΔCt
	GAPDH	FPRL1	
Total brain	23,53	32,58	9,05
Hippocampus	23,44	32,12	8,68
Cerebral cortex	23,7	32,27	8,57

TABLE 5: Homologues to the GCGR receptor

Gene name	Identical residues (%)	Similar residues (%)
GIPR	50	63
GLP1R	49	64
GLP2R	44	61
VIPR1	41	57
SCTR	39	54
VIPR2	37	53

TABLE 6: Homologues to the FPRL1 receptor

Gene name	Identical residues(%)	Similar residues (%)
FPRL2	71	82
FPR1	68	78
GPR32	40	60
CMKLR1	37	56
C5R1	35	50
GPR44	35	55

5

TABLE 7: Formyl peptide receptor antagonist and agonist

Antagonist	FPR1	FPRL1	FPRL2	Class
Chenodeoxycholic acid	Yes	Yes		Bile acids
Cyclosporin (Cs) H	Yes			
BocPLPLP	Yes			
Agonist	FPR1	FPRL1	FPRL2	Class
Lipoxin A4		Yes		lipoxines
Serum amyloid		Yes		Peptide
MMK-1		Yes		Synthetic peptides
WKYMVm		Yes	Yes	Synthetic peptides
FMLF	Yes	Yes		peptide

TABLE 8: Glucagon receptor antagonist and agonist

Antagonist
Glucagon derivatives such as: [desHis(1)-[Glu(9)]-glucagon-amide [desHis(1), Ala(4), Glu(9)] glucagon amide [desHis(1), D-Ala(4), Glu(9)] glucagon amide [desHis(1), Leu(4), Glu(9)] glucagon amide [desHis(1), D-Leu(4), Glu(9)] glucagon amide
NNC 92-1687
BAY 27-9955
Alkylidene hydrazide derivatives with alkoxyaryl moieties such as: [4-hydroxy-3-cyanobenzoic acid (4-isopropylbenzyloxy-3,5-dimethoxymethylene)hydrazide] 3-cyano-4-hydroxybenzoic acid [1-(2,3,5,6-

tetramethylbenzyl)-1H-indol-4-ylmethylene]hydrazide
non-peptide glucagons receptor antagonists: quinoxalines /pyrrolo[1,2 -a]quinoxalines mercaptobenzimidazoles 2-pyridyl-3,5-diarylpyrroles q uinoline hydrazones 4-phenylpyridines 5-hydroxyalkyl-4-phenylpyridines
Triarylimidazole and triarylpyrrole antagonist such as: 2-(-4-Pyridyl)-5-(4-chlorophenyl)-3-(5-bromo-2-propyloxyphenyl)pyrrole
Agonist
Glucagon

TABLE 9: Sequences for expression inhibitory agent

Accession number	Name	Sequence	SEQ ID NO:
NM_000164	GIPR	AAATGCGCTGCCGGGATTACC	29
NM_000164	GIPR	AACAGGATTCTAGGCGGAAGC	30
NM_000164	GIPR	AACATCAAGTTCCACACACGC	31
NM_000164	GIPR	AACCCAGAGAAGAATGAGGCC	32
NM_000164	GIPR	AACGGGTCCTTCGATATGTAC	33
NM_000164	GIPR	AAGAATGAGGCCTTTCTGGAC	34
NM_000164	GIPR	AAGCTCGGCTTTGAGATCTTC	35
NM_000164	GIPR	AAGGAGGTGCAGTCGGAGATC	36
NM_000164	GIPR	ACCAAAGGCTCATCTTGGAGC	37
NM_000164	GIPR	ACCATACACAATGTGAGAACC	38
NM_000164	GIPR	ACCTGTTCACGTCTTTCATGC	39
NM_000164	GIPR	ACTTCCGCTACTACCTGCTCC	40
NM_001462	FPRL1	AACCTCTTTGGAAGTGTCTTC	41
NM_001462	FPRL1	AAGACTTAGATGAGATAGCGC	42
NM_001462	FPRL1	AAGGGCATGATTAAATCCAGC	43
NM_001462	FPRL1	AATGCCAGTTCCAGCTTCATC	44

NM_001462	FPRL1	ACACGCACAGTCACCACCATC	45
NM_001462	FPRL1	ACAGTCACCACCATCTGTTAC	46
NM_001462	FPRL1	ACATCGTGGTGGACATCAACC	47
NM_001462	FPRL1	ACCACCATCTGTTACCTGAAC	48
NM_001462	FPRL1	ACCCTTGAGTCATATTGAGGC	49
NM_001462	FPRL1	ACCGCTGCATTTGTGTCCTGC	50
NM_001462	FPRL1	ACGGCCACATTACCATTCCTC	51
NM_001462	FPRL1	ACTGCTGTGGTGGCTTCTTTC	52
NM_001462	FPRL1	ACTTCCGAGAGAGACTGATCC	53
NM_001506	GPR32	AAATGGAATGGCTGTACGCAC	54
NM_001506	GPR32	AACTCTGACAATGAGACTGCC	55
NM_001506	GPR32	AACTGCCTCCTTGTCTTCATC	56
NM_001506	GPR32	AAGATGAACTCTTCCGGATGC	57
NM_001506	GPR32	AATGGCTGTACGCACTGCTAC	58
NM_001506	GPR32	ACATTATAGGGACCATTGGCC	59
NM_001506	GPR32	ACCTTTGTGTTCCTCAGCTAC	60
NM_001506	GPR32	ACGCACTGCTACTTGGCGTTC	61
NM_001506	GPR32	ACGTGGTGCTGTTGGTCCATC	62
NM_001506	GPR32	ACTCTGACAATGAGACTGCCC	63
NM_001506	GPR32	ACTGACTGTGGTTATCCTGTC	64
NM_001506	GPR32	ACTGTCTTCCGTATGGCACGC	65
NM_001506	GPR32	ACTTTGCCAGTAACTGCCTCC	66
NM_001736	C5R1	AAACCCATCTGGTGCCAGAAC	67
NM_001736	C5R1	AACACGCTGCGTGTTCCAGAC	68
NM_001736	C5R1	AACGTGTTGACTGAAGAGTCC	69
NM_001736	C5R1	AACTTGGCGGTAGCCGACTTC	70
NM_001736	C5R1	AAGCGGACCATCAATGCCATC	71
NM_001736	C5R1	AAGCTGGACTCCCTGTGTGTC	72
NM_001736	C5R1	AAGGTGTTGTGTGGCGTGGAC	73
NM_001736	C5R1	AATCCCAGAACTTTGGGAGGC	74
NM_001736	C5R1	AATGATGTCCTTCCTGGAGCC	75
NM_001736	C5R1	AATGCCATCTGGTTCCTCAAC	76
NM_001736	C5R1	AATTAGGCTGAGAGCAGTGGC	77

NM_001736	C5R1	ACAAACAGAAACCCGTGTATC	78
NM_001736	C5R1	ACACTATGGCCCAGAAGACCC	79
NM_001736	C5R1	ACACTTCCTTCTAGGGAGCAC	80
NM_001736	C5R1	ACAGAAGTCCATGGAGTTATC	81
NM_001736	C5R1	ACAGAGGGATCTTGTGTACCC	82
NM_001736	C5R1	ACAGGACATTCTCATCACCAC	83
NM_001736	C5R1	ACATCAACTGCTGCATCAACC	84
NM_001736	C5R1	ACATGTACGCCAGCATCCTGC	85
NM_001736	C5R1	ACCATACCCTCCTTCCTGTAC	86
NM_001736	C5R1	ACCATCAATGCCATCTGGTTC	87
NM_001736	C5R1	ACCTTAGCTAACTAACTCTCC	88
NM_001736	C5R1	ACGCTGCGTGTTCCAGACATC	89
NM_001736	C5R1	ACGTCCATTGTACAGCATCAC	90
NM_001736	C5R1	ACTAACTCTCCTCCATGTTGC	91
NM_001736	C5R1	ACTACAGCCACGACAAACGGC	92
NM_001736	C5R1	ACTTCCTTCTAGGGAGCACCC	93
NM_002030	FPRL2	AAAGACTGATTCGCTCTTTGC	94
NM_002030	FPRL2	AACACCATCTGTTACCTGAAC	95
NM_002030	FPRL2	AACCCAACAAGCTCCTTGGCC	96
NM_002030	FPRL2	AACCTGGCCCTAGCTGACTTC	97
NM_002030	FPRL2	AACCTGTTTGTCAGTGTCTAC	98
NM_002030	FPRL2	AACGTGTTCATTACCATGGCC	99
NM_002030	FPRL2	AAGACTGATTCGCTCTTTGCC	100
NM_002030	FPRL2	AAGAGGGTGATGACGGGACTC	101
NM_002030	FPRL2	AAGGTCTTTCTGATCCTCCAC	102
NM_002030	FPRL2	ACACACCACTTCTGCTTCACC	103
NM_002030	FPRL2	ACACCACTTCTGCTTCACCTC	104
NM_002030	FPRL2	ACACCATCTGTTACCTGAACC	105
NM_002030	FPRL2	ACACGCACAGTCAACACCATC	106
NM_002030	FPRL2	ACAGCTGCCTCAACCCAATTC	107
NM_002030	FPRL2	ACAGTCAACACCATCTGTTAC	108
NM_002030	FPRL2	ACAGTCTGCTATGGGATCATC	109
NM_002030	FPRL2	ACATGATTAAATCCAGCCGTC	110

NM_002030	FPRL2	ACCACATGATTAAATCCAGCC	111
NM_002030	FPRL2	ACCATCATTGCTCTGGACCGC	112
NM_002030	FPRL2	ACCGCTGTATTTGTGTCCTGC	113
NM_002030	FPRL2	ACCTGATCACCATCATTGCTC	114
NM_002030	FPRL2	ACCTGTTTGTCAGTGTCTACC	115
NM_002030	FPRL2	ACGGTGCCTATGTCCATCATC	116
NM_002030	FPRL2	ACTGATTCGCTCTTTGCCCAC	117
NM_002030	FPRL2	ACTGCTGTAGAGAGGTTGAAC	118
NM_002030	FPRL2	ACTTCTCTTTCAGTGCCATCC	119
NM_002062	GLP1R	AAATGCAGACTTGCCAAGTCC	120
NM_002062	GLP1R	AAATGGCGAGAATACCGACGC	121
NM_002062	GLP1R	AACCGGACCTTCGATGAATAC	122
NM_002062	GLP1R	AACCTCAGCCAAACACAGAGC	123
NM_002062	GLP1R	AACCTGTTTGCATCCTTCATC	124
NM_002062	GLP1R	AAGAGACTCTCTTAGGGAAAC	125
NM_002062	GLP1R	AAGAGAGACATTGCCTCCACC	126
NM_002062	GLP1R	AAGCAGCCTCCTAATTTGATC	127
NM_002062	GLP1R	AAGCTGTTTACAGAGCTCTCC	128
NM_002062	GLP1R	AAGGGAAGCTGTTTGTGTGTC	129
NM_002062	GLP1R	AAGTCCACGCTGACACTCATC	130
NM_002062	GLP1R	AAGTGGATGTATAGCACAGCC	131
NM_002062	GLP1R	AATCTCATGTGCAAGACAGAC	132
NM_002062	GLP1R	AATGGCGAGAATACCGACGCC	133
NM_002062	GLP1R	AATGGGCAATTCTGACTTCTC	134
NM_002062	GLP1R	AATTTCGGAAGAGCTGGGAGC	135
NM_002062	GLP1R	ACAATGGGCAATTCTGACTTC	136
NM_002062	GLP1R	ACACACACACATACATCCTGC	137
NM_002062	GLP1R	ACACACATACATCCTGCTTTC	138
NM_002062	GLP1R	ACACATACATCCTGCTTTCCC	139
NM_002062	GLP1R	ACACGTTAGGAATGTCCAGAC	140
NM_002062	GLP1R	ACAGAGCTCTCCTTCACCTCC	141
NM_002062	GLP1R	ACAGCAGCACTGCAGATAGCC	142
NM_002062	GLP1R	ACATACATCCTGCTTTCCCTC	143

NM_002062	GLP1R	ACATGGCTATCCTAGAGAGGC	144
NM_002062	GLP1R	ACCAGGAACTCCAACATGAAC	145
NM_002062	GLP1R	ACCTGTTTGCATCCTTCATCC	146
NM_002062	GLP1R	ACCTTCGATGAATACGCCTGC	147
NM_002062	GLP1R	ACGCACTCTCCTTCTCTGCTC	148
NM_002062	GLP1R	ACTACTGGCTCATTATCCGGC	149
NM_002062	GLP1R	ACTCATGAGGTCATCTTTGCC	150
NM_002062	GLP1R	ACTCCAACATGAACTACTGGC	151
NM_002062	GLP1R	ACTGCACCAGGAACTACATCC	152
NM_002062	GLP1R	ACTGGCTCATTATCCGGCTGC	153
NM_002062	GLP1R	ACTTGCCAAGTCCACGCTGAC	154
NM_002062	GLP1R	ACTTTATCTGTGACCACACGC	155
NM_002980	SCTR	AAAGTCATGTACACCGTGGGC	156
NM_002980	SCTR	AACGAGAAGCGGCACTCCTAC	157
NM_002980	SCTR	AACGCATCCATCTGGTGGATC	158
NM_002980	SCTR	AACTAGCCCTTGGCTCATTCC	159
NM_002980	SCTR	AAGAAGTGGCAGCAATGGCAC	160
NM_002980	SCTR	AAGACCAGTGCCTGCAGGAAC	161
NM_002980	SCTR	AAGAGCAAGACCAGTGCCTGC	162
NM_002980	SCTR	AAGCTGAAAGTCATGTACACC	163
NM_002980	SCTR	AAGCTGGTCATGGTGCTGTTC	164
NM_002980	SCTR	AAGGCCTCTACCTTCACACAC	165
NM_002980	SCTR	AAGTCAGCCATTATAAGCGCC	166
NM_002980	SCTR	AAGTGGCAGCAATGGCACCTC	167
NM_002980	SCTR	AATGGCACCTCCGTGAGTTCC	168
NM_002980	SCTR	AATGGTTCCTTGTTCCGAAAC	169
NM_002980	SCTR	AATGTGAACGACTCTTCCAAC	170
NM_002980	SCTR	ACAAGAGGAAATGAAGTCAGC	171
NM_002980	SCTR	ACACTCCTCGCCATCTCCTTC	172
NM_002980	SCTR	ACAGGATGGCTGGTCAGAAAC	173
NM_002980	SCTR	ACATCGTCTTCGCCTTCTCCC	174
NM_002980	SCTR	ACCTGCAGGACCAGCATCATC	175
NM_002980	SCTR	ACCTGTTCGTGTCCTTCATCC	176

NM_002980	SCTR	ACGACTCTTCCAACGAGAAGC	177
NM_002980	SCTR	ACTCTTCCAACGAGAAGCGGC	178
NM_002980	SCTR	ACTGCACTCGCAACTACATCC	179
NM_002980	SCTR	ACTTCATCAAGGACGCCGTGC	180
NM_003382	VIPR2	AAACACAAAGCCTGCAGTGGC	181
NM_003382	VIPR2	AAACTGTTTCAAGCCCTCCTC	182
NM_003382	VIPR2	AAAGCAGCAGTGTCCAGAGAC	183
NM_003382	VIPR2	AAAGCCTGATCTCACATCTGC	184
NM_003382	VIPR2	AAATCAACAGGAGGGCAGCCC	185
NM_003382	VIPR2	AACCTGTTCCTGTCCTTCATC	186
NM_003382	VIPR2	AACTGTTTCAAGCCCTCCTCC	187
NM_003382	VIPR2	AAGCAGCAGTGTCCAGAGACC	188
NM_003382	VIPR2	AAGCAGGACCCAGTGGTCAAC	189
NM_003382	VIPR2	AAGCTGGTTGTCCACTAAACC	190
NM_003382	VIPR2	AAGGACGACGTTCTCTACTCC	191
NM_003382	VIPR2	AAGGAGGAAATGTGGAAACGC	192
NM_003382	VIPR2	AAGGCCATTTATACCCTGGGC	193
NM_003382	VIPR2	AAGTCCACGCTCCTGCTTATC	194
NM_003382	VIPR2	ACAAACGACCACAGTGTGCCC	195
NM_003382	VIPR2	ACAAGCTCATCCCTGGACTTC	196
NM_003382	VIPR2	ACACATCCTGTCAGTGTCACC	197
NM_003382	VIPR2	ACAGGAAGCATAATTCTGTGC	198
NM_003382	VIPR2	ACAGGGTTTCACCATGTTAGC	199
NM_003382	VIPR2	ACAGTGTCTCTCTGATGTCTC	200
NM_003382	VIPR2	ACATCCACCTGAACCTGTTCC	201
NM_003382	VIPR2	ACATGGTGTTTGCCGTGTTTC	202
NM_003382	VIPR2	ACCAGTCTCAGTACAAGAGGC	203
NM_003382	VIPR2	ACCATGTTAGCCAGGATGGTC	204
NM_003382	VIPR2	ACCCAGTGGTCAACAGGTGTC	205
NM_003382	VIPR2	ACCGCACATGTGCCACTGTTC	206
NM_003382	VIPR2	ACCGGTTGCTGGGATACAAAC	207
NM_003382	VIPR2	ACCTCTCCACACAGGTGTTCC	208
NM_003382	VIPR2	ACCTGTTCCTGTCCTTCATCC	209

NM_003382	VIPR2	ACGGATGGTCAGAGACGTTCC	210
NM_003382	VIPR2	ACGTTAGGACCAGGAGAAATC	211
NM_003382	VIPR2	ACTCCGTCAAGCTGGTTGTCC	212
NM_003382	VIPR2	ACTGCACCAGGAATTACATCC	213
NM_003382	VIPR2	ACTGTTTCAAGCCCTCCTCCC	214
NM_004072	CMKLR1	AACATGGTCTGGTTCCTCAAC	215
NM_004072	CMKLR1	AACCTCCTAGAGCTCCACCAC	216
NM_004072	CMKLR1	AACCTGGCAGTGGCAGATTTC	217
NM_004072	CMKLR1	AACTTCCTTCTCATCCACAAC	218
NM_004072	CMKLR1	AAGAACCTCTTTAGCATCCAC	219
NM_004072	CMKLR1	AAGAAGACAGTGAACATGGTC	220
NM_004072	CMKLR1	AAGAAGTTCAAGGTGGCCCTC	221
NM_004072	CMKLR1	AAGATCAGCAACTTCCTTCTC	222
NM_004072	CMKLR1	AAGTGAAGATACAGGCCACTC	223
NM_004072	CMKLR1	AAGTTCAAGGTGGCCCTCTTC	224
NM_004072	CMKLR1	AATCCATATCACCTATGCCGC	225
NM_004072	CMKLR1	AATTTATGCTTCTTGGGAGGC	226
NM_004072	CMKLR1	ACAACTTCAGCCTGTCCACAC	227
NM_004072	CMKLR1	ACACACTCAACCTCCTAGAGC	228
NM_004072	CMKLR1	ACACTCAACCTCCTAGAGCTC	229
NM_004072	CMKLR1	ACAGCCATGTGCAAGATCAGC	230
NM_004072	CMKLR1	ACAGCTTGCTACCTCACCATC	231
NM_004072	CMKLR1	ACAGTGAACATGGTCTGGTTC	232
NM_004072	CMKLR1	ACATGCTGTGTTCCATACAGC	233
NM_004072	CMKLR1	ACATGGTCTGGTTCCTCAACC	234
NM_004072	CMKLR1	ACATGGTGGTGACTGTCACCC	235
NM_004072	CMKLR1	ACATGTTCACCAGCGTCTTCC	236
NM_004072	CMKLR1	ACCATCATCAGCTCTGACCGC	237
NM_004072	CMKLR1	ACCTATGCCGCCATGGACTAC	238
NM_004072	CMKLR1	ACCTCACCATCGTGTGCAAAC	239
NM_004072	CMKLR1	ACCTGGCAGTGGCAGATTTCC	240
NM_004072	CMKLR1	ACCTTCTTCCTCTGCTGGTGC	241
NM_004072	CMKLR1	ACTCTCTCAACCCAGGGACAC	242

NM_004072	CMKLR1	ACTGCCCTTGCCATTGCCAAC	243
NM_004246	GLP2R	AAACAGGCATGTCTGAGAGAC	244
NM_004246	GLP2R	AAACGACTCGGAAGTGGGCTC	245
NM_004246	GLP2R	AAACTCCACTGCACGCGCAAC	246
NM_004246	GLP2R	AAATGTCTCTGTACCCTGCCC	247
NM_004246	GLP2R	AACCTTGCAGCTGATGTACAC	248
NM_004246	GLP2R	AACGGGACATTTGATCAGTAC	249
NM_004246	GLP2R	AAGCAAGTTACAGGATCCCTC	250
NM_004246	GLP2R	AAGCTCTCGGAAGGAGATGGC	251
NM_004246	GLP2R	AAGCTGCAGCCCTCACTTAAC	252
NM_004246	GLP2R	AAGGACGTCGTCTTCTACAAC	253
NM_004246	GLP2R	AAGGAGATGGCGCTGAGAAGC	254
NM_004246	GLP2R	AAGGCTGAGCTGCGGAAATAC	255
NM_004246	GLP2R	AATACTGGGTCCGCTTCTTGC	256
NM_004246	GLP2R	AATCAACACTGGTCCTCATTC	257
NM_004246	GLP2R	AATGAGAATGGGTGGATGTCC	258
NM_004246	GLP2R	ACAACTCTTACTCCAAGAGGC	259
NM_004246	GLP2R	ACCAGTCCTCTCTCCTTCCAC	260
NM_004246	GLP2R	ACCCATGATGCTCTGTGTAAC	261
NM_004246	GLP2R	ACCCTGCCCTTCATACTTACC	262
NM_004246	GLP2R	ACCTTGCAGCTGATGTACACC	263
NM_004246	GLP2R	ACGGATATTTGGCAGGATGAC	264
NM_004246	GLP2R	ACGTGGACCGTTATGCCTTGC	265
NM_004246	GLP2R	ACTCCGAATGCTCCGAGAACC	266
NM_004246	GLP2R	ACTCCTTCTCTCTTATCTCCC	267
NM_004246	GLP2R	ACTCGGAAGTGGGCTCAGTAC	268
NM_004246	GLP2R	ACTCTGGTCCTGCTGGTTTCC	269
NM_004246	GLP2R	ACTTGGCAGACGATAGAGAAC	270
NM_004624	VIPR1	AAAGACTTGGCCCTCTTCGAC	271
NM_004624	VIPR1	AAAGCAGATACCTCACCCTGC	272
NM_004624	VIPR1	AACAGGAATCAAGAGCTGCCC	273
NM_004624	VIPR1	AACCCAAGGACTGAGGGACTC	274
NM_004624	VIPR1	AACTACATCCACATGCACCTC	275

NM_004624	VIPR1	AACTCAGTCATTAGACTCCTC	276
NM_004624	VIPR1	AACTCCTCACTGTGGTGGATC	277
NM_004624	VIPR1	AAGACCGGCTACACCATTGGC	278
NM_004624	VIPR1	AAGAGTGACAGCAGTCCATAC	279
NM_004624	VIPR1	AAGATGGTCTTTGAGCTCGTC	280
NM_004624	VIPR1	AAGATGTGGGACAACCTCACC	281
NM_004624	VIPR1	AAGCCTGAAGTGAAGATGGTC	282
NM_004624	VIPR1	AAGGTCACCAGCACCAACACC	283
NM_004624	VIPR1	AAGTCTCCCTGGTCTGACCAC	284
NM_004624	VIPR1	AAGTGAAGATGGTCTTTGAGC	285
NM_004624	VIPR1	AAGTGAGAGAGATGGGAGCTC	286
NM_004624	VIPR1	AATGAGAAGGCAGCCACCAGC	287
NM_004624	VIPR1	AATGAGACAATAGGCTGCAGC	288
NM_004624	VIPR1	AATGTAAGCCGCAGCTGCACC	289
NM_004624	VIPR1	ACACCTATCTTAGTGGTTCCC	290
NM_004624	VIPR1	ACACCTCTGCCAGAAGATCCC	291
NM_004624	VIPR1	ACACTCCTAGAGAACGCAGCC	292
NM_004624	VIPR1	ACAGAAAGCAGATACCTCACC	293
NM_004624	VIPR1	ACAGCAGTCCATACTCAAGGC	294
NM_004624	VIPR1	ACAGCTATCCTGAGCCTGTTC	295
NM_004624	VIPR1	ACATCATGTTCGCCTTCTTTC	296
NM_004624	VIPR1	ACATTCACCATGGTGTGGACC	297
NM_004624	VIPR1	ACCGGCTACACCATTGGCTAC	298
NM_004624	VIPR1	ACCGGTGGATCCTCAAACAAC	299
NM_004624	VIPR1	ACCTCACCCTGCTACACATAC	300
NM_004624	VIPR1	ACCTCCATCTTGGTAAACTTC	301
NM_004624	VIPR1	ACCTCTTCATATCCTTCATCC	302
NM_004624	VIPR1	ACGCAGGTTTCCATGCTGACC	303
NM_004624	VIPR1	ACTAGGCTCAGAGATGTGCAC	304
NM_004624	VIPR1	ACTCAGCTTCCTACCCACACC	305
NM_004624	VIPR1	ACTCAGTCATTAGACTCCTCC	306
NM_004624	VIPR1	ACTGAAGATGCAGCTCACTAC	307
NM_004624	VIPR1	ACTGAGGGACTCTGAAGCCTC	308

NM_004624	VIPR1	ACTGCAACAGGCTTGTGCAAC	309
NM_004624	VIPR1	ACTGCACGCGGAACTACATCC	310
NM_004624	VIPR1	ACTTTCATCCTGACTCTGCCC	311
NM_004778	GPR44	AAACTCTTGAGATCTTGGTCC	312
NM_004778	GPR44	AAACTGCACTCCTCCATCTTC	313
NM_004778	GPR44	AAAGGGAACAGTGAGGTGCCC	314
NM_004778	GPR44	AAAGTATCACCAGGGTGCCGC	315
NM_004778	GPR44	AACAGTGAGTTAAAGCAGTGC	316
NM_004778	GPR44	AACATGTTCGCCAGCGGCTTC	317
NM_004778	GPR44	AACCCTAGGCATCACATGCTC	318
NM_004778	GPR44	AACTCGTAATAGACTTCCCAC	319
NM_004778	GPR44	AACTCTAAGACTACAGCACAC	320
NM_004778	GPR44	AACTTGCACCTCTGACCTATC	321
NM_004778	GPR44	AAGGTTTGAGAAGCACTGTTC	322
NM_004778	GPR44	AAGTGCTTCCAAGGCAGAAGC	323
NM_004778	GPR44	AAGTTGAATGGGCACAGCAAC	324
NM_004778	GPR44	AATCCCAAGATCTGTGCAGCC	325
NM_004778	GPR44	AATGCTTACTGCGCTAGACGC	326
NM_004778	GPR44	ACAATGTGCTGCTCCTGAACC	327
NM_004778	GPR44	ACAGGGTCTGCACTCTAACCC	328
NM_004778	GPR44	ACCACCTTCTGCAAACTGCAC	329
NM_004778	GPR44	ACCAGCATCCGCTACATCGAC	330
NM_004778	GPR44	ACCAGCCTGGCCTTCTTCAAC	331
NM_004778	GPR44	ACCTATCACTTCCACTGCACC	332
NM_004778	GPR44	ACCTTCTGCAAACTGCACTCC	333
NM_004778	GPR44	ACCTTGATGTGCCTGTGAATC	334
NM_004778	GPR44	ACGGTGCCCTATTTCGTGTTC	335
NM_004778	GPR44	ACTCACACGCGAAAGTATCAC	336
NM_004778	GPR44	ACTGCGCTAGACGCTTCATCC	337
NM_004778	GPR44	ACTGCTGTGTTTGAGCTCTGC	338

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EXAMPLES

EXAMPLE 1: GPCRs decrease amyloid beta 1-42 levels

To identify novel drug targets that change the APP processing, a stable cell line overexpressing APP, Hek293 APPwt, is transduced with adenoviral cDNA libraries and the resulting amyloid beta 1-42 levels are detected via ELISA. This stable cell line is created after transfection of Hek293 cells with the APP770wt cDNA cloned in pcDNA3.1 and selection with G418 during 3 weeks. At this time point colonies are picked and stable clones are expanded and tested for their secreted amyloid beta peptide levels.

The assay is performed as follows. Cells seeded in collagen-coated plates at a cell density of 15000 cells/well (384 well plate) in DMEM 10%FBS, are infected 24 h later with 1 □l or 0.2 □l of adenovirus (corresponding to an average multiplicity of infection (MOI) of 120 and 24 respectively). The following day, the virus is washed away and DMEM 25 mM Hepes 10%FBS is added to the cells. Amyloid beta peptides are allowed to accumulate during 24h. The ELISA plate is prepared by coating the capture antibody (JRF/cAbeta42/26) (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium) overnight in buffer 42 (table 2) at a concentration of 2,5 μg/ml. The excess capture antibody is washed away the next morning with PBS and the ELISA plate is then blocked overnight with casein buffer (table 2) at 4°C. Upon removal of the blocking buffer, 30 μl of the sample is transferred to the ELISA plate and incubated overnight at 4°C. After extensive washing with PBS-Tween20 and PBS, 30 μl of the horse reddish peroxidase (HRP) labeled detection antibody (Peroxidase Labeling Kit, Roche), JRF/AbetaN/25-HRP (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium) is diluted 1/5000 in buffer C (table 2) and

added to the wells for another 2h. Following the removal of excess detection antibody by a wash with PBS-Tween20 and PBS, HRP activity is detected via addition of luminol substrate (Roche), which is converted into a chemiluminescent signal by the HRP enzyme.

In order to validate the assay, the effect of adenoviral overexpression with random titre of two clinical PS1 mutants and BACE on amyloid beta 1-42 production is evaluated in the Hek293 APPwt cells. As is shown in Figure 2, all constructs induce amyloid beta 1-42 levels as expected.

An adenoviral cDNA library containing almost all GPCRs was constructed as follows. DNA fragments covering the full coding region of the GPCRs, are amplified by PCR from a pooled placental and fetal liver cDNA library (InvitroGen). All fragments are cloned into our proprietary adenoviral vector (see US 6,340,595) and subsequently adenoviruses are made harboring the corresponding cDNAs. During the screening of the adenoviral GPCR library in the Hek293 APPwt cells, FPRL1 and GCGR were identified as modulators of APP processing. (see figure 3). 3 adenoviruses harboring different clones of GCGR score above the cut-off value, while 1 adenovirus harboring the FPRL1 cDNA scores positive. These results indicate that overexpression of FPRL1 and GCGR lead to increased levels of amyloid beta 1-42 peptides in the conditioned medium of Hek293 APPwt cells, showing that both GPCRs modulate APP processing.

The stimulatory effect of FPRL1 and GCGR is confirmed upon re-screening of the viruses with a known titer (viral particles/ml), as determined by quantitative real time PCR. FPRL1 and GCGR viruses are infected at MOIs ranging from 2 to 1250 and the experiment is performed as described above. Amyloid beta 1-42 levels are 4 fold higher compared to the negative controls for Ad5/FPRL1 and Ad5/GCGR clones at MOI 1250 (figure 4A). In addition, the effect of FPRL1 and GCGR

on amyloid beta 1-40 and 1-x levels are checked under similar conditions as above. The respective ELISA's are performed as described above, except that the following antibodies were used: for the amyloid beta 1-40 ELISA, the capture and detection antibody are respectively JRF/cAbeta40/10 and JRF/AbetaN/25-HRP (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium), while for the amyloid beta 1-x ELISA (x ranges from 19-42) the capture and detection antibodies are JRF/AbetaN/25 and 4G8-HRP, respectively (obtained respectively from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium and from Signet, USA). The amyloid beta 1-x ELISA is used for the detection of amyloid peptides with a variable C-terminus (amyloid beta 1-37; 1-38; 1-39; 1-40; 1-42). The results of these experiments clearly show an increase of amyloid beta 1-40 and 1-x species upon transduction of FPRL1 and GCGR (figure 4B and 4C). These are surprising results according to what is known about FPRL1 and its relation to amyloid peptides. Classic studies suggested that the N-formyl group was a crucial determinant of ligand binding and because bacterial and mitochondrial proteins are the only sources in nature, it was widely thought that these receptors evolved to mediate trafficking of phagocytes to sites of bacterial invasion or tissue damage. However, over the past five years, data from several groups have indicated that these receptors might act in a more complex manner, since a large number of non-formylated peptide ligands have now been identified. FPRL1 is known as a GPCR that has both endogenous peptide and lipid (lipoxin A4) ligands. At least three host-derived polypeptides are identified as ligands for this receptor, which are all associated with amyloidogenic diseases: serum amyloid A, prion protein fragment106-126 and amyloid beta 1-42.

The relevance of FPRL1 to Alzheimer's disease is in its relation to the inflammatory aspects of the disease and is underscored by FPRL1 being a chemotactic receptor for amyloid beta 1-42, which induces monocyte migration and activation. In brain tissue of AD patients, mononuclear phagocytes that surround or infiltrate the plaques express high levels of FPRL1. In addition, FPRL1 can promote the cellular uptake of amyloid beta 1-42 by rapid internalisation into the cytoplasmic compartment in the form of amyloid beta 1-42-FPRL1 complexes. Moreover, amyloid fibrils and aggregates are accumulated in macrophages in an FPRL1-mediated fashion. Hence, following roles in the mechanisms of amyloid beta 1-42 amyloid aggregation and degradation are suggested: intracellular fibril formation of amyloid beta 1-42 and/or removal from the extracellular environment and endoproteolysis of amyloid beta 1-42.

However, the relationship between the FPRL1 receptor and amyloid beta production/secretion has never been studied before, and the finding that FPRL1 increases amyloid beta 1-42 production/secretion in the conditioned medium of infected cells is completely novel.

EXAMPLE 2: Identification of close relatives of FPRL1 and GCGR.

The amino acid sequence of the human GCGR receptor was used as query in a BLAST search against all the human GPCRs in order to find its closest homologues. Table 6 shows the 5 closest homologues of the glucagon receptor. Using ClustalW an alignment was constructed showing the degree of homology between the GCGR and its closest homologues, the GLPR1 and GLPR2 (fig. 7).

The amino acid sequence of the human FPRL1 receptor was used as query in a BLAST search against all the human GPCRs in order to find its closest homologues. Table 5 shows the 5

closest homologues of the FPRL1 receptor. Using ClustalW an alignment was constructed showing the degree of homology between the GCGR and its closest homologues, the FPR1 and FPRL2 (fig 8).

EXAMPLE 3: Functional analysis of GPCR receptors in HEK293 cells by reporter gene analysis.

All GPCRs share a common architecture of 7 transmembrane domains, an extracellular N-terminus and an intracellular C-terminus. The major signal transduction cascades activated by GPCRs are initiated by the activation of heterotrimeric G-proteins (Wess(1998)). In addition, minor signal transduction pathways that are G-protein independent exist (Marinissen and Gutkind (2001)). Heterotrimeric G-proteins are built from three different proteins; the Gα, Gβ and Gγ subunits. The signal transduction cascade starts with the activation of the receptor by an agonist. Transformational changes in the receptor are then translated down to the G-protein. The G-protein dissociates into the G_α subunit and the $G_{\beta\gamma}$ subunit. Both subunits dissociate from the receptor and are both capable of initiating different cellular responses. Best known are the cellular effects that are initiated by the G_α subunit. It is for this reason that G-proteins are categorized by their G_α subunit. The G-proteins are divided into four groups: G_s , $G_{i/o}$, G_q and $G_{12/13}$. Each of these G-proteins is capable of activating an effector protein, which results in changes in second messenger levels in the cell. The changes in second messenger level are the triggers that make the cell respond to the extracellular signal in a specific manner. Cellular responses range over a plethora of possibilities, from changes in cell shape to the transcriptional activation of genes. The two most important second messengers in the cell are cAMP and Ca^{2+}. The α-

subunit of the G_s class of G-proteins is able to activate adenylyl cyclase, resulting in an increased turnover from ATP to cAMP. The α-subunit of $G_{i/o}$ G-proteins does exactly the opposite and inhibits adenylyl cyclase activity resulting in a decrease of cellular cAMP levels. Together, these two classes of G-proteins regulate the second messenger cAMP. Ca^{2+} is regulated by the α-subunit of the G_q class of G-proteins. Through the activation of phospholipase C phosphatidylinositol 4,5-bisphosphate (PIP2) from the cell membrane are hydrolyzed to inositol 1,4,5-trisphosphate and 1,2-diacylglycerol, both these molecules act as second messengers. Inositol 1,4,5-trisphosphate binds specific receptors in the endoplasmatic reticulum, resulting in the opening of Ca^{2+} channels and release of Ca^{2+} in the cytoplasm.

Receptor activation can be measured by several different techniques. Usually these measurements detect the levels of second messengers either directly by ELISA or radioactive technologies or indirectly by reporter gene analysis. Reporter gene technology consists of an easily detectable gene, such as luciferase or β-galactosidase under the regulation of a promoter that responds to the cellular level of second messengers.

For the measurement of changes in cAMP levels we use a luciferase gene placed under the control of a minimal promoter regulated by cAMP responsive elements (CRE). In the cell, cAMP binds to the regulatory subunit of protein kinase A (PKA) and by forcing the subunit to dissociate from the catalytic subunit cAMP activates PKA. cAMP responsive element binding protein (CREB) is one of the many substrates of PKA and is therefore phosphorylated by PKA. Upon phosphorylation, CREB translocates to the nucleus and binds to CRE DNA sequences in promoter regions, initiating transcription of downstream genes. Activation of G_s by a GPCR will thus result in an increase in luciferase activity

when the reporter gene construct is present in the same cell as the receptor.

A similar reporter gene is constructed for the measurement of changes in intracellular Ca^{2+} levels. This reporter makes use of the Ca^{2+} dependent activation of the transcription factor NF-AT (nuclear factor activated T-cells). To activate this transcription factor $Ca^{2+}_{@}$ must activate calcineurin, which in turn acts as a phosphatase for NF-AT. The dephosphorylated form of NF-AT translocates to the nucleus and binds specific promoter elements. Binding of NF-AT to these cis-acting elements drives the transcription of a downstream gene, in our case the luciferase gene.

We have constructed both reporter gene constructs into an adenoviral vector. By doing so we can make an adenovirus and use this virus to introduce the reporter gene construct into our assay cells with the purpose to measure GPCR activation.

Adenoviruses are constructed harboring the luciferase gene under the control of a minimal promoter with respectively CRE elements or NF-AT responsive elements. HEK293 cells are transduced with adenoviruses containing GPCRs and either the CRE reporter or the NF-AT reporter,.

In general cells are plated in a 96 well plate at a density of 10,000 cells per well in Dulbeco´s modified Eagles medium (DMEM) supplemented with 10% fetal calf serum (FCS). After the cells are firmly attached, GPCR or control viruses expressing eGFP and LacZ, are added to the cells with a MOI of 50. Subsequently reporter virus is added at an MOI of 400. The cells are incubated for 18 h with the virus before the virus is washed away and the medium replaced with DMEM, 5% FCS. The cells are left for an additional 24 h before they are treated with increasing amounts of agonist (glucagon or fMLF) for a period of 6 h after which the cells are lysed and the luciferase activity is measured using the steady

light kit from Packard according to the manufacturer's protocol.

Stimulation of GCGR with increasing amounts of glucagon shows a dose dependent increase in luciferase activity indicating that activation of the glucagon receptor results in an increase of intracellular cAMP (fig. 5A) and NF-AT (fig. 5B). This result indicates that the glucagon receptor couples in HEK 293 cells to G_s and G-protein giving rise to increased intracellular Ca^{2+}- levels.

Stimulation of FPRL1 with increasing amounts of fMLF shows a dose dependent decrease in luciferase activity indicating that activation of the FPRL1 receptor results in an decrease of intracellular cAMP (fig. 5C). Forskoline (10 μM) was added simultaneously with the ligand to increase the basal cAMP content of the cells so that a larger window of detection is created. This result indicates that the formyl petpide receptor couples in HEK 293 cells to G_{i1-3} or G_o.

EXAMPLE 4: Effect on amyloid beta peptide production by an agonist activated G protein coupled receptor.

Whereas overexpression of GPCRs results in constitutive signalling, the activity of endogenous GPCRs is normally modulated by binding of natural occurring agonists or antagonists. Because this is why they are good drug targets, it is of great value for future therapeutic applications to show that amyloid beta levels can be modulated by the agonists or antagonists of the GPCRs.

Therefore, the effect of the fMLF (agonist for FPRL1) and glucagon peptides (agonist for GCGR) on amyloid beta levels are evaluated in the Hek293 APPwt cells. Hek293 APPwt cells are transduced with respectively Ad5/empty, Ad5/GCGR and Ad5/FPRL1 at an MOI of 50 during 24 h. Viruses are washed away and fresh medium containing respectively 5nM glucagon and 1mM fMLF is added to the cells. 24h later, the

conditioned medium is assayed in the amyloid beta 1-42 ELISA as described in example 1. It is observed that the addition of 5nM glucagon to cells transduced with Ad5/GCGR results in a 2 fold increase of amyloid beta 1-42 levels compared to un-stimulated cells transduced with either Ad5/GCGR or Ad5/empty, indicating that an agonist of GCGR is able to modulate amyloid beta 1-42 levels (figure 6A). Similarly, stimulating Hek293 APPwt cells, that are transduced with Ad5/FPRL1, with 1mM fMLF yields an increase in the amyloid beta 1-42 levels compared to un-stimulated cells transduced with either Ad5/FPRL1 or Ad5/empty, indicating that an agonist of FPRL1 is able to modulate amyloid beta 1-42 levels (figure 6B).

In addition, antagonists for FPRL1 and GCGR are tested to evaluate whether inhibiting the GPCRs results in a decrease of the amyloid beta 1-42 levels. Hek293 APPwt cells are infected with respectively Ad5/empty, Ad5/GCGR and Ad5/FPRL1 during 24 h. Viruses are washed away and fresh medium containing respectively 5nM glucagon +/- antagonist and 1mM fMLF +/- antagonist are added to the cells. 24h later, the conditioned medium is assayed in the amyloid beta 1-42 ELISA as described in example 1.

EXAMPLE 5: Expression of GPCRs in the human brain

Upon identification of a modulator of APP processing, it is of the highest importance to evaluate whether the modulator is expressed in the tissue and the cells of interest. This can be achieved by measuring the RNA and/or protein levels. In recent years, RNA levels are being quantified through real time PCR technologies, whereby the RNA is first transcribed to cDNA and then the amplification of the cDNA of interest is monitored during a PCR reaction. The amplification plot and the resulting Ct value are indicators for the amount of RNA present in the sample.

Determination of the levels of household keeping genes allows the normalization of RNA levels of the target gene between different RNA samples, represented as delta Ct values.

To assess whether the GPCRs of the invention are expressed in the human brain, real time PCR with GAPDH specific primers and specific primers for the GPCRs (Table 3) is performed on a dilution series of human total brain, human cerebral cortex, and human hippocampal total RNA (BD Biosciences). GAPDH was detected with a Taqman probe, while for the other GPCRs SybrGreen was used. In short, 40 ng of RNA is transcribed to DNA using the MultiScribe Reverse Transcriptase (50 U/µl) enzyme (Applied BioSystems). The resulting cDNA is amplified with AmpliTaq Gold DNA polymerase (Applied BioSystems) during 40 cycles using an ABI PRISM® 7000 Sequence Detection System.

Total brain, cerebral cortex and hippocampal total RNA are analyzed for the presence of GPCR transcripts of table 1 via quantitative real time PCR.

For FPRL1, the obtained Ct values indicate that it is detected in all RNA samples (table 4).

To gain more insight into the specific cellular expression, immunohistochemistry (protein level) and/or *in situ* hybridization (RNA level) are carried out on sections from a human normal and Alzheimer's brain hippocampal, cortical and subcortical structures. These results indicate whether expression occurs in neurons, microglia cells or astrocytes. The comparison of diseased tissue with healthy tissue, teaches us whether the GPCRs of the invention are expressed in the diseased tissue and whether their expression level is changed compared to the non-pathological situation.

EXAMPLE 6: Amyloid beta production in rat primary neuronal cells

In order to investigate whether GPCRs of the invention affect amyloid beta production in a real neuron, human or rat primary hippocampal or cortical neurons are transduced with adenoviruses containing the GPCRs. Amyloid beta levels are determined by ELISA and mass spectrometry analysis (see EXAMPLE 6). Since rodent APP genes carry a number of mutations in APP compared to the human sequence, they produce less amyloid beta 1-40 and 1-42. In order, to achieve detectable amyloid beta levels, co-transduction with human wild type APP or human Swedish mutant APP (which enhances Abeta production) cDNA is necessary.

Human primary neurons are purchased from Cellial Technologies, France. Rat primary neuron cultures are prepared from brain of E18-E19-day-old fetal Sprague Dawley rats according to Goslin and Banker (Culturing Nerve cells, second edition, 1998 ISBN 0-262-02438-1). Briefly, single cell suspensions obtained from the hippocampus or cortices are prepared. The number of cells is determined (only taking into account the living cells) and cells are plated on poly-L-lysine-coated plastic 96-well plates in minimal essential medium (MEM) supplemented with 10% horse serum. The cells are seeded at a density between 30,000 and 60,000 cells per well (i.e. about 100,000 - 200,000 cells/cm^2, respectively). After 3-4 h, culture medium was replaced by 150 µl serum-free neurobasal medium with B27 supplement (GIBCO BRL). Cytosine arabinoside (5 µM) was added 24 h after plating to prevent nonneuronal (glial) cell proliferation.

Neurons are used at day 5-7 after plating. Before adenoviral transduction, 150 µl conditioned medium of these cultures is transferred to the corresponding wells in an empty 96-well plate and 50 µl of the conditioned medium returns to the cells. The remaining 100 µl/well is stored at

37°C and 5% CO_2. Both hippocampal and cortical primary neuron cultures are coinfected with the crude lysate of virus containing the cDNAs of the GPCRs, and human wild type APP or human Swedish mutant APP, at different MOIs, ranging from 100 to 3000. Sixteen to twenty-four hours after transduction, virus is removed and cultures are washed with 100 µl pre-warmed fresh neurobasal medium. After removal of the wash solution, the remaining 100 µl of the stored conditioned medium is transferred to the corresponding cells. From now on, cells accumulate amyloid beta in the conditioned medium and its concentration is determined by amyloid beta 1-42 and Amyloid beta x-42 specific ELISA's (see EXAMPLE 1). The conditioned media are collected 24, 48 and 96 hours after exchanging virus-containing medium by stored conditioned medium.

EXAMPLE 7: Amyloid beta peptides profiling in conditioned medium of GPCRs of the invention infected HEK293 APP770wt cells and rat primary neuronal cells using Mass Spectrometry

To specify how APP processing is exactly modulated by GPCRs of the present invention a mass spectrometry analysis is carried out on the conditioned medium of cells overexpressing the latter GPCRs or cells into which the activity of the endogenous GPCR is inhibited with its antagonist, to identify the inhibited amyloid beta peptide species.

T25 flasks (Cellstar, Greiner Bio-One) are coated with collagen (5 µg/ml) for 4h at 37°C. After replacement of the collagen by medium (DMEM from GIBCO with 10% FBS from ICN), HEK293 APP770wt cells are seeded at a density of 3.10^6 cells per flask. Cells are grown overnight at 37°C, 10% CO_2. Next day, cells are infected with the crude lysate of virus containing the cDNAs of the GPCRs at the appropriate MOI. The

cells are incubated at 37°C, 10% CO_2. After 12 to 24 hours, the cell culture medium is removed by aspiration and 3 ml of fresh medium (DMEM, 0.2% FBS, 1X ITS from GIBCO) is added to the cells. 24 hours later, the conditioned medium is harvested. Protease inhibitors are added immediately and the samples are kept on ice in Falcon tubes until further processing.

Of each sample, 850 µl of the conditioned medium is transferred to an eppendorf tube in triplet. After rigorously vortexing the Protein G Sepharose beads, 5 µl of the slurry is added to each tube, together with 1 µg of specific antibody e.g. 4G8 or JRF/cAbeta42/26 (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium). Tubes are rotated overnight at 4°C and centrifuged for 10 min. All centrifuge steps are at 13200 rpm at 4°C. After aspiration of the supernatant, beads are washed twice by adding 850 µl of wash buffer (10 mM Tris-HCl (pH 8.0) containing 0.1% n-octylglucoside, 150 mM NaCl, 0.025% sodium azide) and centrifuging for 10 min. After a final wash step with 850 µl of 10 mM Tris-HCl (pH 8.0), cells are centrifuged for 10 min and supernatant is removed completely. Dry pellets are stored at -80°C until further analysis.

A saturated solution of matrix (alpha-cyano-hydroxy-cinnamic acid, HCCA) is prepared in 500 µl acetonitrile by vortexing. After adding 400 µl water and 100 µl 1% trifluoroacetic acid, the tube is vortexed for 3 min. This results in 50% acetonitrile/0.1% TFA matrix containing elution buffer. 3.5 µl of this elution buffer is added to 5 µl of thawed dry beads and sonicated for 1 min in a water bath at room temperature. The samples are briefly spun (30 s) at maximal speed (14.000 rpm).

One µl of eluted sample is directly spotted on a ground stainless steel MALDI target plate. Samples are allowed to

air dry until crystallization of sample. The target plate is inserted into the MALDI-TOF-TOF mass spectrometer and measurements are performed according to the MALDI-TOF instructions. The resulting spectra are calibrated using a standard curve acquired using a mixture of several standard peptides obtained from Sigma. These standard peptides are in the mass range of 1200 - 3200 Da.

EXAMPLE 8: Ligand screen for GPCRs

Reporter gene screen

Mammalian cells such as HEK293 or CHO-K1 cells are either stably transfected with a plasmid harboring the luciferase gene under the control of a cAMP dependent promoter (CRE elements) or transduced with an adenovirus harboring a luciferase gene under the control of a cAMP dependent promoter. In addition reporter constructs can be used with the luciferase gene under the control of a Ca^{2+} dependent promoter (NF-AT elements) or a promoter that is controlled by activated NF-κB. These cells, expressing the reporter construct, are then transduced with an adenovirus harboring the cDNA of the GPCR of the present invention. 40 h after transduction the cells are treated with an agonist for the receptor (table 7 and 8) and screened against a large collection of reference compounds comprising peptides (LOPAP, Sigma Aldrich), lipids (Biomol, TimTech), carbohydrates (Specs), natural compounds (Specs, TimTech), and small chemical compounds (Tocris). Compounds, which decrease the agonist induced increase in luciferase activity, are considered to be antagonists or inverse agonists for the GPCR they are screened for. These compounds are screened again for verification and screened against their effect on secreted amyloid beta peptide levels.

In addition, cells expressing the NF-AT reporter gene can be transduced with an adenovirus harboring the cDNA

encoding the α-subunit of G_{15} or chimerical Gα subunits. G_{15} is a promiscuous G protein of the G_q class that couples to many different GPCRs and as such re-directs their signaling towards the release of intracellular Ca^{2+} stores. The chimerical G alpha subunits are members of the G_s and $G_{i/o}$ family by which the last 5 C-terminal residues are replaced by those of Gαq, these chimerical G-proteins also redirect cAMP signaling to Ca^{2+} signaling.

FLIPR screen

Mammalian cells such as HEK293 or CHO-K1 cells are stably transfected with a expression plasmid construct harboring the cDNA of a GPCR of the present invention. Cells are seeded and grown until sufficient stable cells can be obtained. Cells are loaded with a Ca^{2+} dependent fluorophore such as Fura3 or Fura4. After washing away the excess of fluorophore the cells are screened against a large collection of reference compounds comprising peptides (LOPAP, Sigma Aldrich), lipids (Biomol, TimTech), carbohydrates (Specs), natural compounds (Specs, TimTech), and small chemical compounds (Tocris) by simultaneously adding an agonist (Table 7) and a compound to the cells. As a reference just the agonist is added. Activation of the receptor is measured as an almost instantaneously increase in fluorescence due to the interaction of the fluorophore and the Ca^{2+} that is released. Compounds that reduce or inhibit the agonist induced increase in fluorescence are considered to be antagonists or inverse agonists for the receptor they are screened against. These compounds will be screened again to measure the secreted amyloid beta peptide.

AequoScreen

CHO cells, stably expressing Apoaequorin are stably transfected with a plasmid construct harboring the cDNA of a GPCR. Cells are seeded and grown until sufficient stable cells can be obtained. The cells are loaded with

coelenterazine, a cofactor for apoaequorin. Upon receptor activation intracellular Ca^{2+} stores will be emptied and the aequorin will react with the coelenterazine in a light emitting process. The emitted light is a measure for receptor activation. The CHO, stable expressing both the apoaequorin and the receptor are screened against a large collection of reference compounds comprising peptides (LOPAP, Sigma Aldrich), lipids (Biomol, TimTech), carbohydrates (Specs), natural compounds (Specs, TimTech), and small chemical compounds (Tocris) by simultaneously adding an agonist and a compound to the cells. As a reference just the agonist is added. Activation of the receptor is measured as an almost instantaneously light flash due to the interaction of the apoaequorin, coelenterazine and the Ca^{2+} that is released. Compounds that reduce or inhibit the agonist induced increase in light are considered to be antagonists or inverse agonists for the receptor they are screened against. These compounds will be screened again for verification and secreted amyloid beta levels.

In addition, CHO cells stable expressing the apoaequorin gene are stably transfected with a plasmid construct harboring the cDNA encoding the α-subunit of G_{15} or chimerical G_α subunits. G_{15} is a promiscuous G protein of the G_q class that couples to many different GPCRs and as such redirect their signaling towards the release of intracellular Ca2+ stores. The chimerical G alpha subunits are members of the G_s and $G_{i/o}$ family by which the last 5 C-terminal residues are replaced by those of $G_{\alpha q}$, these chimerical G-proteins also redirect cAMP signaling to Ca2+ signaling.

Screening for compounds that bind to the polypeptides of the present invention

Compounds are screened for binding to the polypeptides of the present invention. The affinity of the compounds to the polypeptides is determined in a displacement experiment.

In brief, the polypeptides of the present invention are incubated with a labeled (radiolabeled, fluorescent labeled) ligand that is known to bind to the polypeptide and with an unlabeled compound. The displacement of the labeled ligand from the polypeptide is determined by measuring the amount of labeled ligand that is still associated with the polypeptide. The amount associated with the polypeptide is plotted against the concentration of the compound to calculate IC_{50} values. This value reflects the binding affinity of the compound to its target, i.e. the polypeptides of the present invention. Strong binders have an IC50 in the nanomolar and even picomolar range. Compounds that have an IC_{50} of at least 10 micromol or better (nmol to pmol) are applied in beta amyloid secretion assay to check for their effect on the beta amyloid secretion and processing. The polypeptides of the present invention can be prepared in a number of ways depending on whether the assay will be run on cells, cell fractions or biochemically, on purified proteins.

Receptor ligand binding study on cell surface

The receptor is expressed in mamalian cells (HEK293, CHO, COS7) cells by adenovirally transducing the cells (see US 6,340,595). The cells are incubated with both labeled ligand (iodinated, tritiated, or fluorescent) and the unlabeled compound at various concentrations, ranging from 10 pM to 10μM (3 hours at 4°C.: 25 mM HEPES, 140 mM NaCl, 1 mM $CaCl_2$, 5 mM $MgCl_2$ and 0.2% BSA, adjusted to pH 7.4). Reactions mixtures are aspirated onto PEI-treated GF/B glass filters using a cell harvester (Packard). The filters are washed twice with ice cold wash buffer (25 mM HEPES, 500 mM NaCl, 1 mM $CaCl_2$, 5 mM $MgCl_2$, adjusted to pH 7.4). Scintillant (MicroScint-10; 35 μl) is added to dried filters and the filters counted in a (Packard Topcount) scintillation counter. Data are analyzed and plotted using Prism software (GraphPad Software, San Diego, Calif.). Competition curves

are analyzed and IC_{50} values calculated. If 1 or more datapoints do not fall within the sigmoidal range of the competition curve or close to the sigmoidal range the assay is repeated and concentrations of labeled ligand and unlabeled compound adapted to have more data points close to or in the sigmoidal range of the curve.

Receptor ligand binding studies on membrane preparations

Membranes preparations are isolated from mammalian cells (HEK293, CHO, COS7) cells overexpressing the receptor is done as follows: Medium is aspirated from the transduced cells and cells are harvested in 1 x PBS by gentle scraping. Cells are pelleted (2500 rpm 5 min) and resuspended in 50 mM Tris pH 7.4 (10 x 10E6 cells/ml). The cell pellet is homogenized by sonicating 3 x 5 sec (UP50H; sonotrode MS1; max amplitude: 140 µm; max Sonic Power Density: 125W/cm^2). Membrane fractions are prepared by centrifuging 20 min at maximal speed (13000 rpm ~15 000 to 20 000g or rcf). The resulting pellet is resuspended in 500 µl 50 mM Tris pH 7.4 and sonicated again for 3 x 5 sec. The membrane fraction is isolated by centrifugation and finally resuspended in PBS. Binding competition and derivation of IC_{50} values are determined as described above.

EXAMPLE 9: Inhibition of the GPCR mediated effect on amyloid beta production via knock down of the GPCR expression levels.

The effect of an antagonist can be mimicked through the use of siRNA based strategies, which result in decreased expression levels of the targeted protein. Adenoviral mediated siRNA or knock down constructs based upon the sequences shown in table 9, are constructed as described in WO03020931. Cell lines (e.g. Hek293, SH-SY5Y, IMR-32, SK-N-SH, SK-N-MC, H4, CHO, COS, HeLa) stably overexpressing APPwt or not, or rat primary neuronal cells, are transduced with

these adenoviral knock down constructs. 24h later, the adenoviruses are removed and fresh medium is added to the cells. 96 h later, the medium of the cells is refreshed to allow the accumulation of amyloid beta 1-42 peptides. The following day, the conditioned medium of these cells is assayed in the amyloid beta 1-42 ELISA, which is performed as described in example 1.

Claims

1. Method of identifying a compound that changes the amyloid-beta precursor protein processing in a cell, comprising:

(a) providing a host cell expressing a polypeptide having an amino acid sequences selected from the group consisting of SEQ ID NO: 15-28, or a fragment, or a derivative thereof;

(b) determining a first activity level of the polypeptide by measuring the level of one or more second messengers of the polypeptide;

(c) exposing the host cell to a compound;

(d) determining a second activity level of the polypeptide by measuring the level of the second messengers after exposing of the host cell to the compound; and

(e) identifying the compound, by which the second activity level is less than the first activity level.

2. Method according to claim 1, further comprising contacting the host cell with an agonist for the polypeptide before determining the first activity level.

3. The method according to claim 1 or 2, further comprising

(f) contacting a population of mammalian cells expressing a polypeptide having a amino acid sequences selected from the group consisting of SEQ ID NO: 15-28, or a fragment, or a derivative thereof with the compound identified in step (e)

(g) identifying the compound that changes the amyloid-beta precursor protein processing in the cells.

4. Method according to claim 1-3, wherein the polypeptide is FPRL1, as defined by SEQ ID NO: 1.

5. Method according to claim 1-3, wherein the polypeptide is GCGR, as defined by SEQ ID NO: 2.

6. Method according to any of claim 1-5, wherein the activity level is determined with a reporter controlled by a promoter which is responsive to the second messenger.

7. The method according to claim 6, wherein the promoter is a cyclic AMP-responsive promoter, an NF-KB responsive promoter, or a NF-AT responsive promoter.

8. The method according to claims 6 or 7, wherein the reporter is luciferase or β-galactosidase.

9. Method for identifying a compound that changes the amyloid-beta precursor protein processing in a cell, comprising:

(a) contacting one or more compounds with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 15-28, or a derivative, or a fragment thereof,

(b) determining the binding affinity of the compound to the polypeptide,

(c) contacting a population of mammalian cells expressing the polypeptide with the compound that exhibits a binding affinity of at least 10 micromolar, and

(d) identifying the compound that changes the amyloid-beta precursor protein processing in the cells.

10. Method for identifying a compound that changes the amyloid-beta precursor protein processing in cells, comprising:

(a) contacting one or more compounds with a polynucleotide sequence or a vector comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1-14

(b) Ddetermining the binding affinity of the compound to the polynucleotide or to the vector,

(c) contacting a population of mammalian cells expressing the polynucleotide sequence with the compound that exhibits a binding affinity of at least 10 micromolar, and

(d) identifying the compound that changes the amyloid-beta precursor protein processing of the cells.

11. Method according to any of the claims 1-10, wherein the compounds are low molecular weight compounds.

12. Method according to any of the claims 1-10, wherein the compounds are peptides.

13. Method according to any of the claim 1-10, wherein the compounds are lipids.

14. Method according to any of the claim 1-10, wherein the compounds are natural compounds.

15. Method for changing the amyloid-beta precursor protein processing of a cell, comprising inhibiting the biological activity of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 15-28 and fragments, or derivatives thereof by contacting the cell with an expression inhibitory agent that inhibits the

translation in the cell of a polyribonucleotide encoding the polypeptide.

16. Method according to claim 15, wherein the expression inhibitory agent is selected from the group consisting of a antisense RNA, a ribozyme that cleaves the polyribonucleotide, an antisense oligodeoxynucleotide (ODN), a small interfering RNA (siRNA) that is sufficiently homologous to a portion of the polyribonucleotide such that the siRNA is capable of inhibiting the polyribonucleotide that would otherwise cause the production of the polypeptide, and an antibody reactive to the polypeptide.

17. Method according to claim 15, wherein the expression inhibitory agent is a nucleic acid expressing the antisense RNA, a ribozyme that cleaves the polyribonucleotide, an antisense oligodeoxynucleotide (ODN), a siRNA that is sufficiently homologous to a portion of a the polyribonucleotide such that the siRNA is capable of inhibiting the polyribonucleotide that would otherwise cause the production of the polypeptide, or an antibody reactive to the polypeptide

18. Method according to claim 17, wherein the nucleotide is included within a vector.

19. Method according to claim 18, wherein the vector is an adenoviral, retroviral, adeno-associated viral, lentiviral or a sendaiviral vector.

20. Method according to any of claims 16-19, wherein the siRNA comprises a sense strand of 17-23 nucleotides homologous to a nucleotide sequence selected from the group

consisting of SEQ ID NO: 1-14 and an antisense strand of 17-23 nucleotides complementary to the sense strand.

21. Method according to claim 20, wherein the siRNA further comprises a loop region connecting the sense and the antisense strand.

22. Method according to claim 21, wherein the loop region consist of the nucleic acid sequence defined by SEQ ID NO: 339.

23. Method according to any of claim 15-22 wherein the expression inhibitory agent is an antisense RNA, ribozyme, antisense oligodeoxynucleotide, or siRNA comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

24. Polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

25. Polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for use as a medicament.

26. Use of a polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for the manufacture of a medicament for the treatment of a disease involving cognitive impairment.

27. Use according to claim 26, wherein the polynucleotide is a siRNA.

28. Vector comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338

29. Vector comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for use as a medicament.

30. Use of a vector comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for the manufacture of a medicament for the treatment of a disease involving cognitive impairment.

31. Use according to claim 30, wherein the vector encodes a siRNA comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

32. Use according to claims 30 or 31, wherein the vector is an adenoviral, retroviral, adeno-associated viral, lentiviral or a sendaiviral vector.

33. Use according to any of claims 26-27 and 30-32, wherein the disease is Alzheimer's disease.

34. Method for diagnosing a pathological condition involving cognitive impairment or a susceptibility to the condition in a subject comprising:

(a) obtaining a sample of the subject's mRNA corresponding to a nucleic acid selected from the group consisting of SEQ ID NO: 1-14 or a sample of the subject's genomic DNA corresponding to a genomic sequence of a nucleic acid selected from the group consisting of SEQ ID NO: 1-14;

(b) determining the nucleic acid sequence of the subject's mRNA or genomic DNA;

(c) comparing the nucleic acid sequence of the subject's mRNA or genomic DNA with a nucleic acid selected from the group consisting of SEQ ID NO: 1-14 or with a genomic sequence encoding a nucleic acid selected from the group consisting of SEQ ID NO: 1-14 obtained from a database; and

(d) identifying any difference(s) between the nucleic acid sequence of the subject's mRNA or genomic DNA and the nucleic acid selected from the group consisting of SEQ ID NO: 1-14 or the genomic sequence encoding a nucleic acid selected from the group consisting of SEQ ID NO: 1-14 obtained from a database.

35. Method for diagnosing a pathological condition involving cognitive impairment or a susceptibility to the condition in a subject, comprising determining the amount of polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 15-28 in a biological sample, and comparing the amount with the amount of the polypeptide in healthy subjects, wherein an increase of the amount of polypeptide compared to the healthy subjects is indicative of the presence of the pathological condition.

36. Method according to any of claims 36 or 37, wherein the pathological condition is Alzheimer's disease.

Fig. 1 1/15

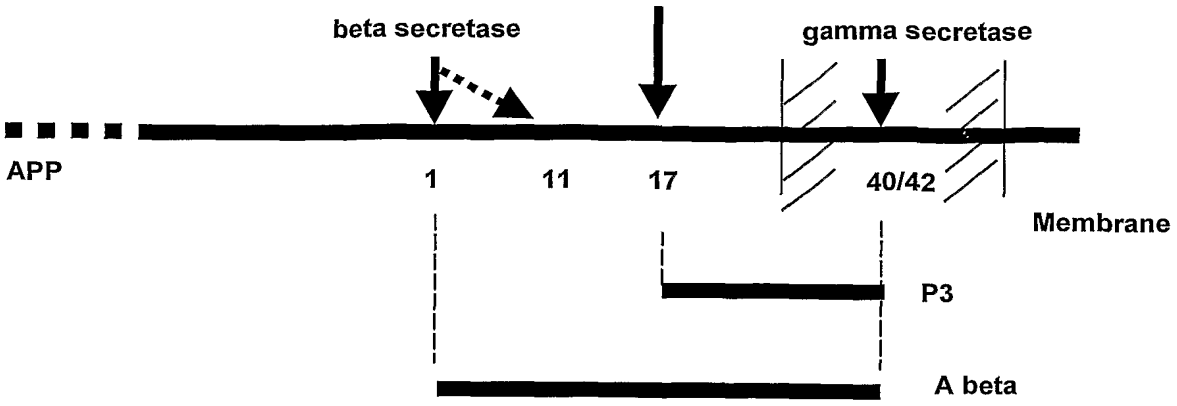
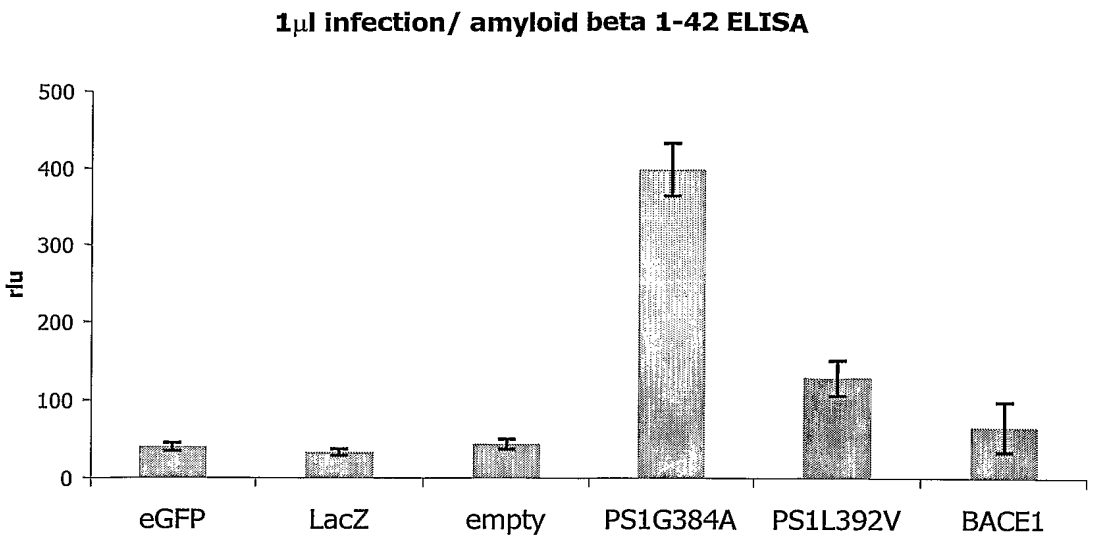

beta secretase
gamma secretase
APP
1
11
17
40/42
Membrane
P3
A beta

Fig. 2 2/15

A


1µl infection/ amyloid beta 1-42 ELISA
500
400
300
200
100
0
rlu
eGFP
LacZ
empty
PS1G384A
PS1L392V
BACE1

B

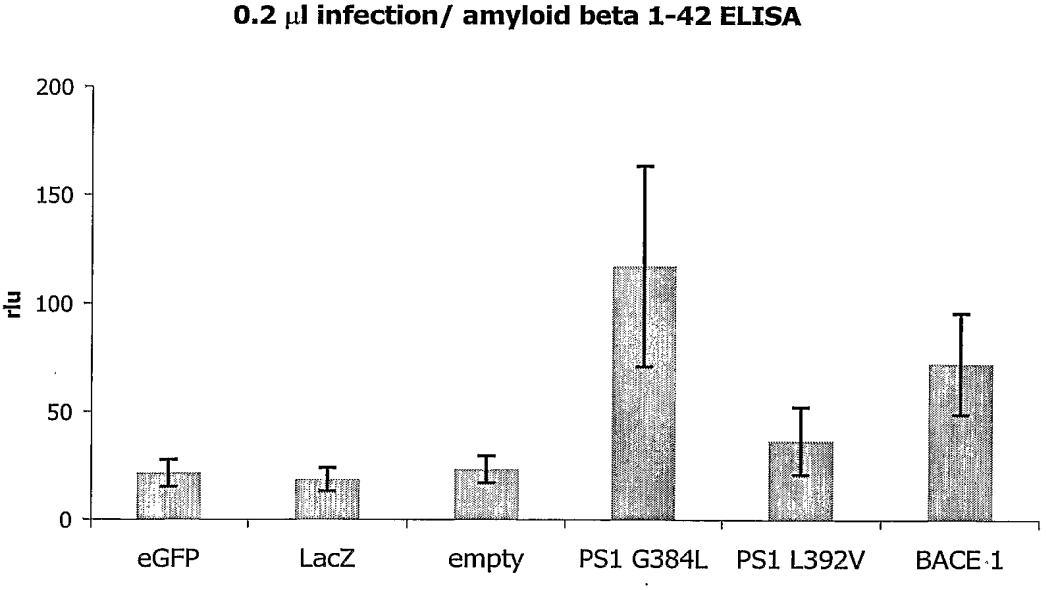

0.2 µl infection/ amyloid beta 1-42 ELISA
200
150
100
50
0
rlu
eGFP
LacZ
empty
PS1 G384L
PS1 L392V
BACE-1

Fig. 3: 3/15

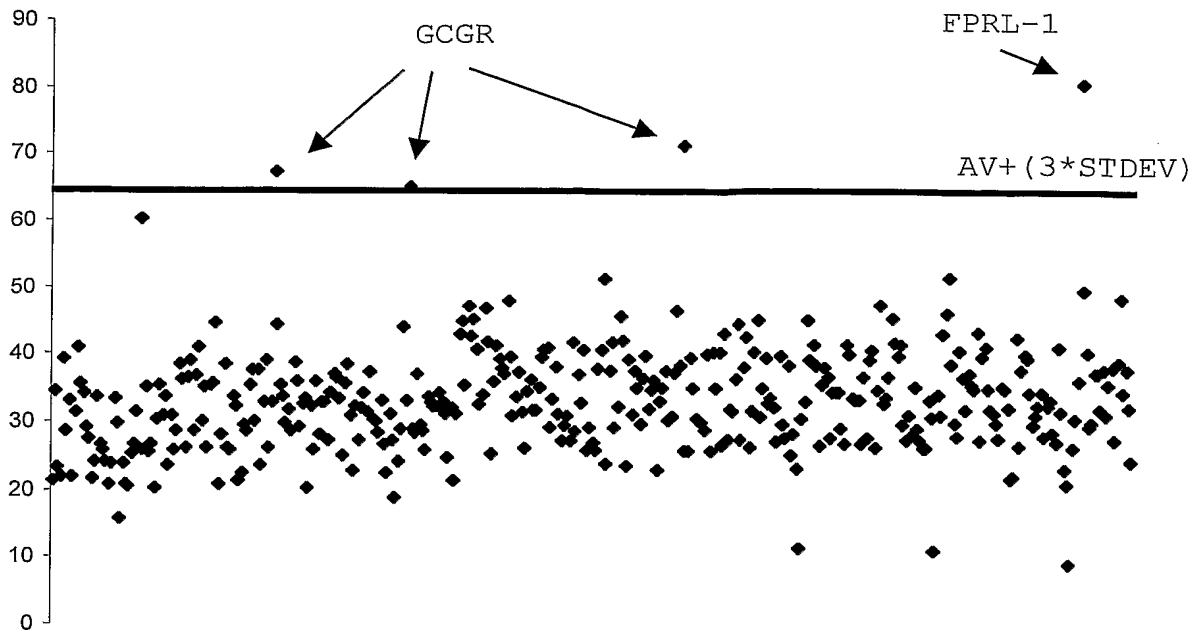
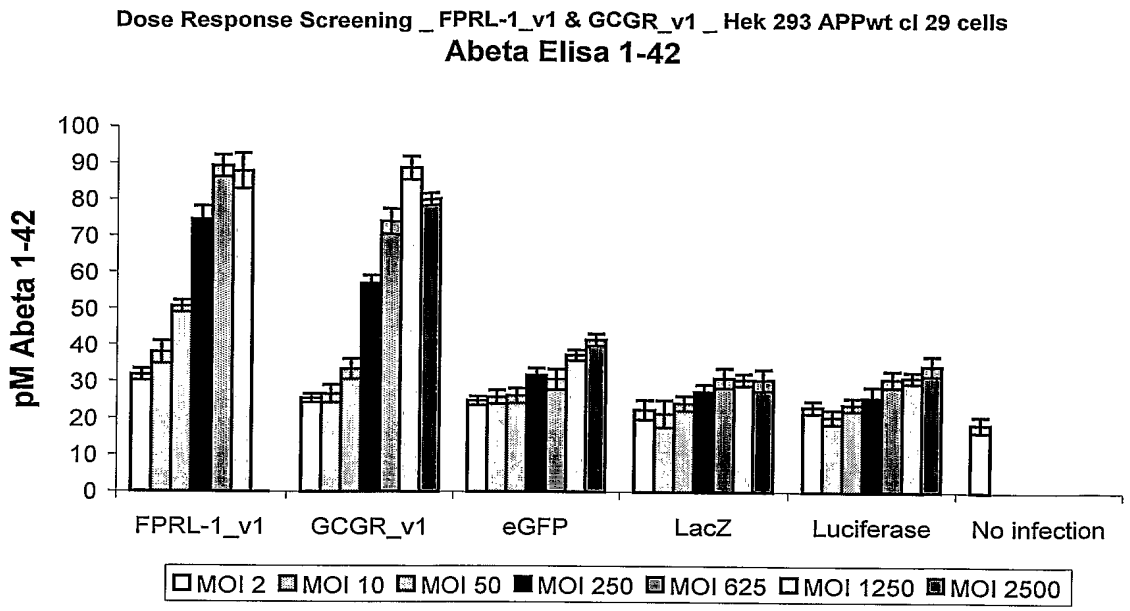


Fig. 4

A


Dose Response Screening _ FPRL-1_v1 & GCGR_v1 _ Hek 293 APPwt cl 29 cells
Abeta Elisa 1-42
pM Abeta 1-42
100
90
80
70
60
50
40
30
20
10
0
FPRL-1_v1
GCGR_v1
eGFP
LacZ
Luciferase
No infection
MOI 2
MOI 10
MOI 50
MOI 250
MOI 625
MOI 1250
MOI 2500

B

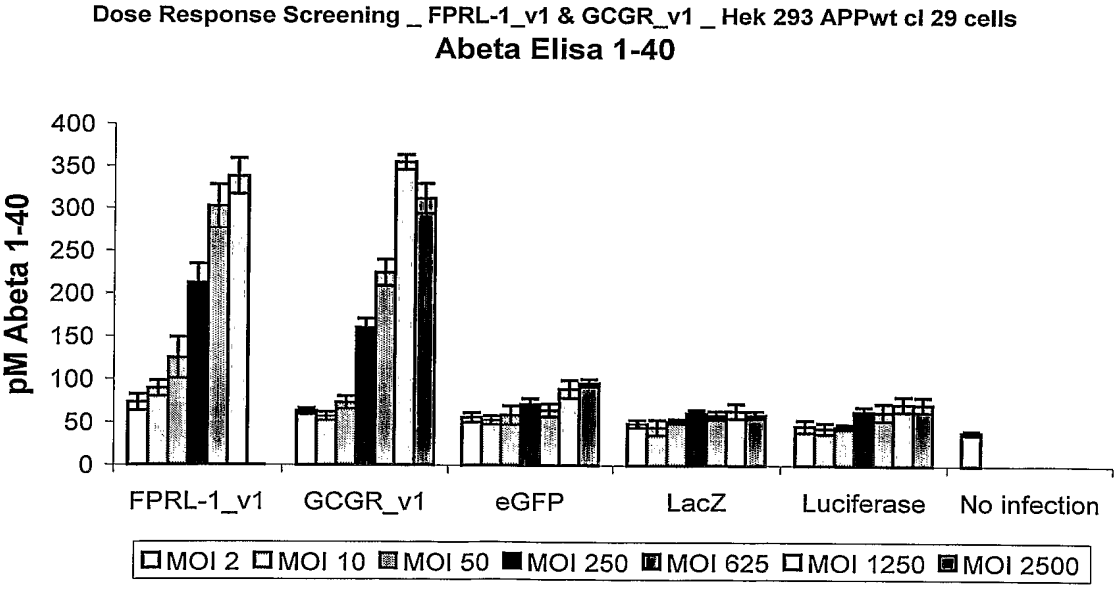

Dose Response Screening _ FPRL-1_v1 & GCGR_v1 _ Hek 293 APPwt cl 29 cells
Abeta Elisa 1-40
pM Abeta 1-40
400
350
300
250
200
150
100
50
0
FPRL-1_v1
GCGR_v1
eGFP
LacZ
Luciferase
No infection
MOI 2
MOI 10
MOI 50
MOI 250
MOI 625
MOI 1250
MOI 2500

Fig. 4

C

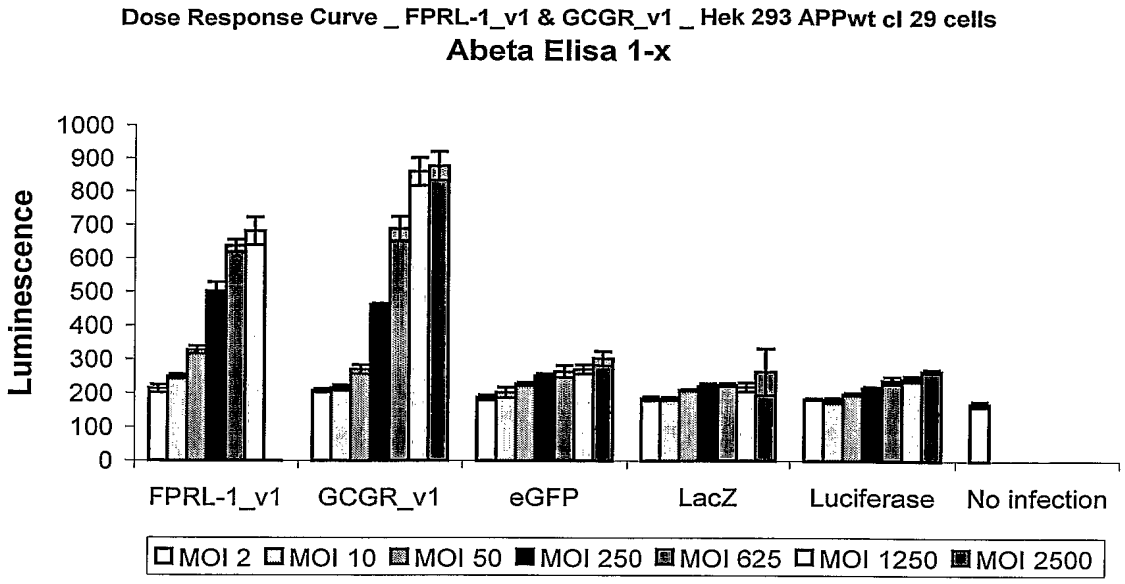
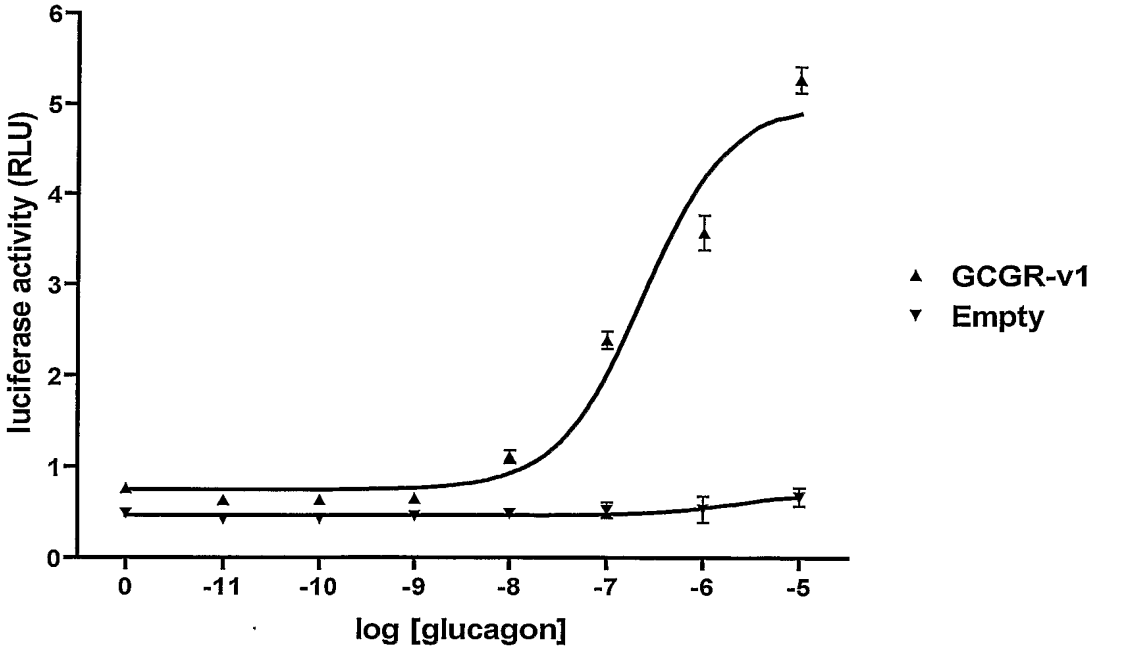

Dose Response Curve _ FPRL-1_v1 & GCGR_v1 _ Hek 293 APPwt cl 29 cells
Abeta Elisa 1-x
Luminescence
1000
900
800
700
600
500
400
300
200
100
0
FPRL-1_v1
GCGR_v1
eGFP
LacZ
Luciferase
No infection
MOI 2
MOI 10
MOI 50
MOI 250
MOI 625
MOI 1250
MOI 2500

Fig. 5 6/15

A


luciferase activity (RLU)
0
1
2
3
4
5
6
0
-11
-10
-9
-8
-7
-6
-5
log [glucagon]
GCGR-v1
Empty

B

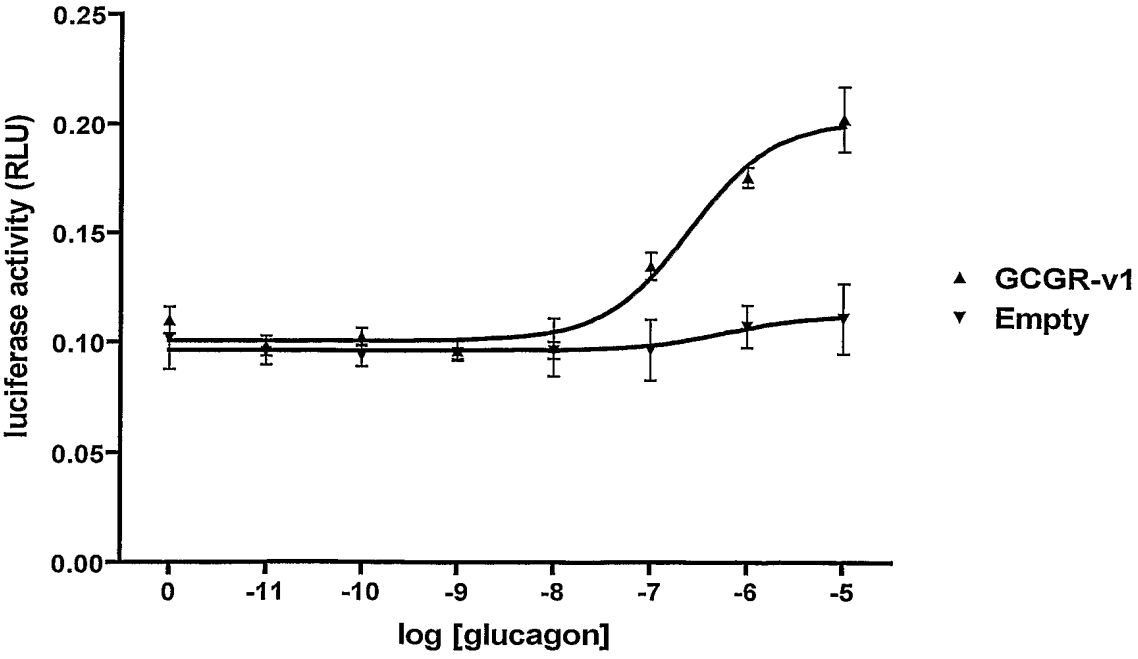

luciferase activity (RLU)
0.00
0.05
0.10
0.15
0.20
0.25
0
-11
-10
-9
-8
-7
-6
-5
log [glucagon]
GCGR-v1
Empty

Fig. 5 7/15

C

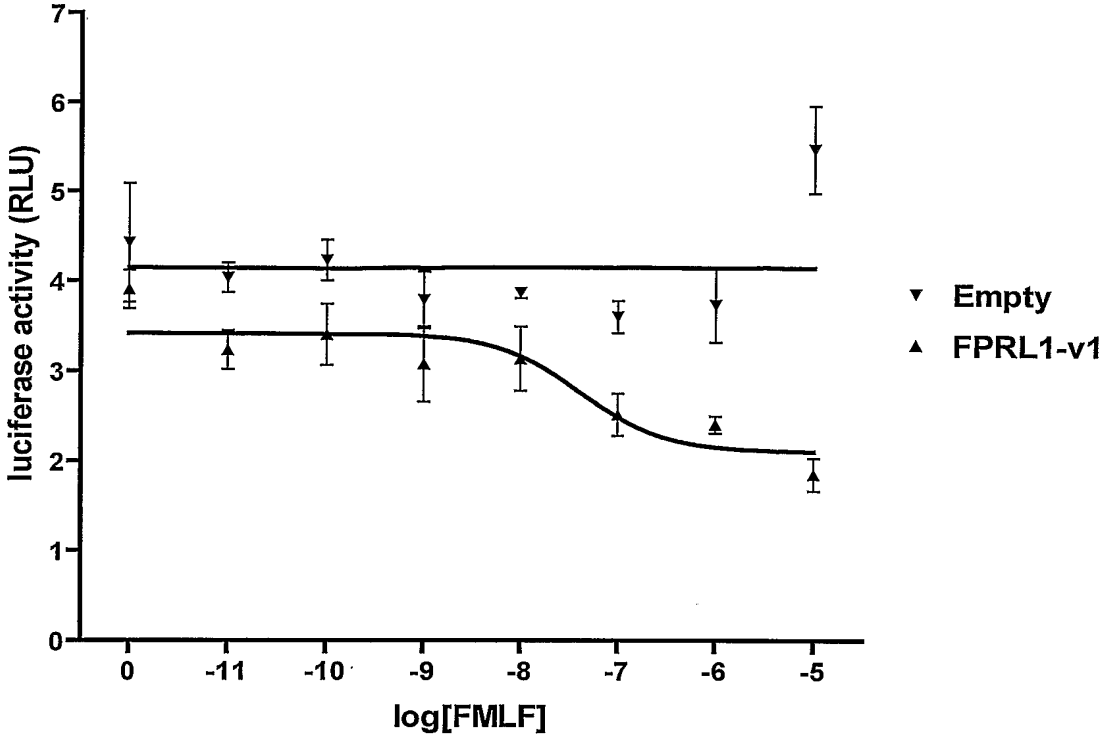

luciferase activity (RLU)
0
1
2
3
4
5
6
7
0
-11
-10
-9
-8
-7
-6
-5
log[FMLF]
Empty
FPRL1-v1

Fig 6

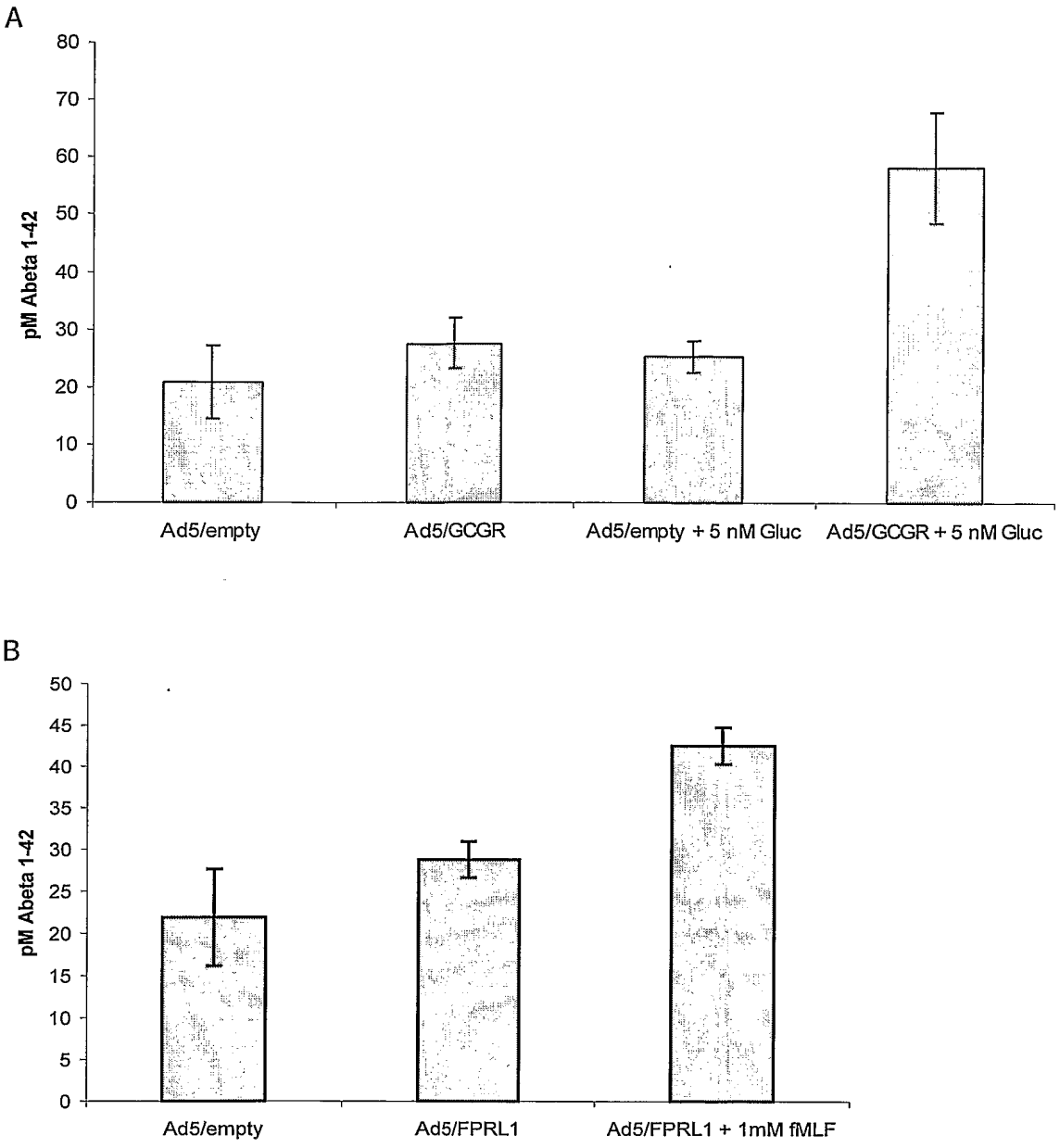

A
pM Abeta 1-42
80
70
60
50
40
30
20
10
0
Ad5/empty
Ad5/GCGR
Ad5/empty + 5 nM Gluc
Ad5/GCGR + 5 nM Gluc
B
pM Abeta 1-42
50
45
40
35
30
25
20
15
10
5
0
Ad5/empty
Ad5/FPRL1
Ad5/FPRL1 + 1mM fMLF

Fig 7 9/15

A.

Clustal W alignment of GCGR peptide sequence with GLP1R peptide sequence.

```
GLP1R   1   MAGAPGPLRL ALLLLGMVGR AGPRPQGATV SLWETVQKWR
EYRRQCQRSL TEDPPPATDL  60
GCGR    1   MPPCQ-PQRP LLLLLLLLAC QPQVPSAQVM DF--LFEKWK
LYGDQCHHNL SLLPPP-TEL  56

GLP1R   61  FCNRTFDEYA CWPDGEPGSF VNVSCPWYLP WASSVPQGHV
YRFCTAEGLW LQKDNSSLPW  120
GCGR    57  VCNRTFDKYS CWPDTPANTT ANISCPWYLP WHHKVQHRFV
FKRCGPDGQW VR-GPRGQPW  115

GLP1R   121 RDLSECEESK RGERSSPEEQ LLFLY----I IYTVGYALSF
SALVIASAIL LGFRHLHCTR  176
GCGR    116 RDASQCQMD- -GEEIEVQKE VAKMYSSFQV MYTVGYSLSL
GALLLALAIL GGLSKLHCTR  173

GLP1R   177 NYIHLNLFAS FILRALSVFI KDAALKWMYS -TAAQQHQWD
GLLSYQDSLS CRLVFLLMQY  235
GCGR    174 NAIHANLFAS FVLKASSVLV IDGLLRTRYS QKIGDDLSVS
TWLSDGAVAG CRVAAVFMQY  233

GLP1R   236 CVAANYYWLL VEGVYLYTLL AFSVFSEQWI FRLYVSIGWG
VPLLFVVPWG IVKYLYEDEG  295
GCGR    234 GIVANYCWLL VEGLYLHNLL GLATLPERSF FSLYLGIGWG
APMLFVVPWA VVKCLFENVQ  293

GLP1R   296 CWTRNSNMNY WLIIRLPILF GIGVNFLIFV RVICIVVSKL
KANLMCKTDI KCRLAKSTLT  355
GCGR    294 CWTSNDNMGF WWILRFPVFL AILINFFIFV RIVQLLVAKL
RARQMHHTDY KFRLAKSTLT  353
```

Fig 7 10/15

```
GLP1R  356 LIPLLGTHEV IFAFVMDEHA RGTLRFIKLF TELSFTSFQG
LMVAILYCFV NNEVQLEFRK  415
GCGR   354 LIPLLGVHEV VFAFVTDEHA QGTLRSAKLF FDLFLSSFQG
LLVAVLYCFL NKEVQSELRR  413

GLP1R  416 SWERWRLEHL HIQ------- RDSSMKPLKC PTSSLSSGAT
AGSSMYTATC QASCS-----  463
GCGR   414 RWHRWRLGKV LWEERNTSNH RASSSPGHGP PSKELQFGRG
GGSQDSSAET PLAGGLPRLA  473

GLP1R  463 ---- 463
GCGR   474 ESPF 477
```

B.

Clustal W alignment of GCGR peptide sequence with GLP2R peptide sequence.

```
GLP2R  1   MKLGSSRAGP GRGSAGLLPG VHELPMGIPA PWGTSPLSFH
RKCSLWAPGR PFLTLVLIVS  60
GCGR   1   ---------- ---------- -------MPP ---------- -----
CQPQR PLLLLILLLA  18

GLP2R  61  IK-QVT-GSL LEETTRKWAQ YKQACLR--D LLKEPSGIFC
NGTFDQYVCW PHSSPGNVS-  115
GCGR   19  CQPQVPSAQV MDFLFEKWKL YGDQCHHNLS LLPPPTELVC
NRTFDKYSCW PDTPANTTAN  78

GLP2R  116 VPCPSYLPWW SEESSGRAYR HCLAQGTWQT IENATDIWQD
DSECSEN-HS FKQNVDRYAL  174
GCGR   79  ISCPWYLPWH HKVQHRFVFK RCGPDGQWVR GPRG-QPWRD
ASQCQMDGEE IEVQKEVAKM  137
```

Fig 7 11/15

```
GLP2R  175 LSTLQLMYTV GYSFSLISLF LALTLLLFLR KLHCTRNYIH
MNLFASFILR TLAVLVKDVV  234
GCGR   138 YSSFQVMYTV GYSLSLGALL LALAILGGLS KLHCTRNAIH
ANLFASFVLK ASSVLVIDGL  197

GLP2R  235 FYNSYSKRPD NENGWMSYLS EMSTS-CRSV QVLLHYFVGA
NYLWLLVEGL YLHTLLEPTV  293
GCGR   198 LRTRYSQKIG DDLSVSTWLS DGAVAGCRVA AVFMQYGIVA
NYCWLLVEGL YLHNLLGLAT  257

GLP2R  294 LPERRLWPRY LLLGWAFPVL FVVPWGFARA HLENTGCWTT
NGNKKIWWIL RGPMMLCVTV  353
GCGR   258 LPERSFESLY LGIGWGAPML FVVPWAVVKC LFENVQCWTS
NDNMGFWWIL RFPVFLAILI  317

GLP2R  354 NFFIFLKILK LLISKLKAHQ MCFRDYKYRL AKSTLVLIPL
LGVHEIIFSF ITDDQVEGFA  413
GCGR   318 NFFIFVRIVQ LLVAKLRARQ MHHTDYKFRL AKSTLTLIPL
LGVHEVVFAF VTDEHAQGTL  377

GLP2R  414 KLIRLFIQLT LSSFHGFLVA LQYGFANGEV KAELRKYWVR
FLLARHSGCR ACVLGKDFRF  473
GCGR   378 RSAKLFFDLF LSSFQGLLVA VLYCFLNKEV QSELRRRWHR WRLG--
----  ----------  421

GLP2R  474 LGKCPKKLSE GDGAEKLRKL QPSLNSGRLL HLAMRGLGEL
GAQPQQDHAR WPRGSSLSEC  533
GCGR   421 ---------- ----KVLWEE RNTSN----- ---HRASSSP
GHGPPSKELQ FGRGGGSQDS  459

GLP2R  534 SEGDVTMANT MEEILEESEI  553
GCGR   460 S-AETPLAGG LPRLAESPF-  477
```

Fig 7 12/15

C.

Alignment of Glucagon, Glucagon related peptide 1 (GLP1) and Glucagon related peptide (GLP2).

```
glucagon  1  HSQGTFTSDY SKYLDSRRAQ DFVQWLMNT- --- 29
GLP1      1  HAEGTFTSDV SSYLEGQAAK EFIAWLVKGR --- 30
GLP2      1  HADGSFSDEM NTILDNLAAR DFINWLIQTK ITD 33
```

Fig 8 13/15

A.

Clustal W alignment of FPRL1 peptide sequence with FPRL2 peptide sequence.

```
FPRL1  1    METNFSTPLN EYEEVSYESA GYTVLRILPL VVLGVTFVLG
VLGNGLVIWV AGFRMTRTVT  60
FPR1   1    METNSSLPTN ISGGTPAVSA GYLFLDIITY LVFAVTFVLG
VLGNGLVIWV AGFRMTHTVT  60

FPRL1  61   TICYLNLALA DFSFTATLPF LIVSMAMGEK WPFGWFLCKL
IHIVVDINLF GSVFLIGFIA  120
FPR1   61   TISYLNLAVA DFCFTSTLPF FMVRKAMGGH WPFGWFLCKF
VFTIVDINLF GSVFLIALIA  120

FPRL1  121  LDRCICVLHP VWAQNHRTVS LAMKVIVGPW ILALVLTLPV
FLFLTTVTIP NGDTYCTFNF  180
FPR1   121  LDRCVCVLHP VWTQNHRTVS LAKKVIIGPW VMALLLTLPV
IIRVTTVPGK TGTVACTFNF  180

FPRL1  181  ASWGGTPEER LKVAITMLTA RGIIRFVIGF SLPMSIVAIC
YGLIAAKIHK KGMIKSSRPL  240
FPR1   181  SPWTNDPKER INVAVAMLTV RGIIRFIIGF SAPMSIVAVS
YGLIATKIHK QGLIKSSRPL  240

FPRL1  241  RVLTAVVASF FICWFPFQLV ALLGTVWLKE MLFYGKYKII
DILVNPTSSL AFFNSCLNPM  300
FPR1   241  RVLSFVAAAF FLCWSPYQVV ALIATVRIRE LLQ-GMYKEI
GIAVDVTSAL AFFNSCLNPM  299

FPRL1  301  LYVFVGQDFR ERLIHSLPTS LERALSEDSA PTNDTAANSA
SPPAETELQA M 351
```

Fig 8 14/15

```
FPR1    300 LYVFMGQDFR ERLIHALPAS LERALTEDST QTSDTATNST
LPSAEVELQA K 350
```

Identical residues 71% (background black)
Similar residues 82% (background gray)

B. Clustal W alignment of FPRL1 peptide sequence with FPRL2 peptide sequence.

```
FPRL1   1   METNFSTPLN EYEEVSYESA GYTVLRILPL VVLGVTFVLG
VLGNGLVIWV AGFRMTRTVT  60
FPRL2   1   METNFSIPLN ETEEVLPEPA GHTVLWIFSL LVHGVTFVFG
VLGNGLVIWV AGFRMTRTVN  60

FPRL1   61  TICYLNLALA DFSFTATLPF LIVSMAMGEK WPFGWFLCKL
IHIVVDINLF GSVFLIGFIA  120
FPRL2   61  TICYLNLALA DFSFSAILPF RMVSVAMREK WPFASFLCKL
VHVMIDINLF VSVYLITIIA  120

FPRL1   121 LDRCICVLHP VWAQNHRTVS LAMKVIVGPW ILALVLTLPV
FLFLTTVTIP NGDTYCTFNF  180
FPRL2   121 LDRCICVLHP AWAQNHRTMS LAKRVMTGLW IFTIVLTLPN
FIFWTTISTT NGDTYCIFNF  180

FPRL1   181 ASWGGTPEER LKVAITMLTA RGIIRFVIGF SLPMSIVAIC
YGLIAAKIHK KGMIKSSRPL  240
FPRL2   181 AFWGDTAVER LNVFITMAKV FLILHFIIGF TVPMSIITVC
YGIIAAKIHR NHMIKSSRPL  240

FPRL1   241 RVLTAVVASF FICWFPFQLV ALLGTVWLKE MLFYGKYKII
DILVNPTSSL AFFNSCLNPM  300
```

Fig 8

FPRL2 241 RVFAAVVASF FICWFPYELI GILMAVWEKE MILNGKYKII
LVLINPTSSL AFFNSCLNPI 300

FPRL1 301 LYVFVGQDFR ERLIHSLPTS LERALSE--D SAPTNDTAAN
SASPPAETEL QAM 351

FPRL2 301 LYVFMGRNFQ ERLIRSLPTS LERALTEVPD SAQTSNTHTT
SASPPEETEL QAM 353

Identical residues 68% (background black)

Similar residues 78% (background gray)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/10160

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 G01N33/566 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12N C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2003/096260 A1 (PREMACK BRETT ET AL) 22 May 2003 (2003-05-22) abstract paragraph [0006] - paragraph [0012]; figure 1 paragraph [0019] - paragraph [0022] paragraph [0057] - paragraph [0073] paragraph [0092] - paragraph [0111] paragraph [0130] - paragraph [0133] claims 1-24 ----- -/--	1-4, 9-12,14 15-18, 20,21, 23-31, 33-36

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 June 2004	10. 09. 2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Vanhalst, K

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/10160

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 02/29052 A (BAYER AG ; LIOU JIING REN (US)) 11 April 2002 (2002-04-11) abstract page 5, line 1 - page 8, line 6 page 9, line 19 - page 11, line 3 page 21, line 14 - page 27, line 11 page 33, line 6 - page 50, line 27 page 63, line 1 - page 66, line 5 page 69, line 5 - page 70, line 25 examples 1-15 claims 1-71	15-18, 20,21, 23-31, 33-36
A	WO 02/44212 A (DELEERSNIJDER WILLY ; BLOCKX HERMAN (BE); MOOR LUCIE DE (BE); SOLVAY P) 6 June 2002 (2002-06-06) abstract page 1, line 15 - page 4, line 30 page 19, line 25 - page 21, line 20 page 26, line 30 - page 29, line 6 page 29, line 10 - page 31, line 32 examples 1-5 claims 1-43	1-36
A	WO 03/035684 A (BASF LYNX BIOSCIENCE AG ; GRUENEWALD SYLVIA (DE); KUSCHINSKY WOLFGANG) 1 May 2003 (2003-05-01) abstract	1-36
A	LE Y ET AL: "PLEIOTROPIC ROLES OF FORMYL PEPTIDE RECEPTORS" CYTOKINE AND GROWTH FACTOR REVIEWS, OXFORD, GB, vol. 12, no. 1, March 2001 (2001-03), pages 91-105, XP001133719 ISSN: 1359-6101 the whole document	1-36
A	NITSCH R M ET AL: "REGULATION OF PROTEOLYTIC PROCESSING OF THE AMYLOID BETA-PROTEIN PRECURSOR BY FIRST MESSENGERS" ARZNEIMITTEL FORSCHUNG. DRUG RESEARCH, EDITIO CANTOR. AULENDORF, DE, vol. 45, no. 32, 1995, pages 435-438, XP002054765 ISSN: 0004-4172 abstract	1-36

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/10160

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YAZAWA HIROSHI ET AL: "beta amyloid peptide (Abeta42) is internalized via the G-protein-coupled receptor FPRL1 and forms fibrillar aggregates in macrophages" FASEB JOURNAL, vol. 15, no. 13, November 2001 (2001-11), pages 2454-2462, XP002280686 ISSN: 0892-6638 the whole document	1-36
A	WO 01/83553 A (LIND PETER ; SEJLITZ TORSTEN (SE); PARODI LUIS A (US); UPJOHN CO (US)) 8 November 2001 (2001-11-08) abstract	1-36
A	WO 03/065045 A (GOLZ STEFAN ; BAYER AG (DE); GEERTS ANDREAS (DE); BRUEGGEMEIER ULF (DE) 7 August 2003 (2003-08-07) abstract	1-36
E	WO 03/074069 A (GOLZ STEFAN ; BAYER AG (DE); GEERTS ANDREAS (DE); BRUEGGEMEIER ULF (DE) 12 September 2003 (2003-09-12) abstract	1-36
E	WO 03/082314 A (GOLZ STEFAN ; BAYER AG (DE); GEERTS ANDREAS (DE); BRUEGGEMEIER ULF (DE) 9 October 2003 (2003-10-09) abstract	1-36
E	WO 03/080098 A (GOLZ STEFAN ; BAYER AG (DE); GEERTS ANDREAS (DE); BRUEGGEMEIER ULF (DE) 2 October 2003 (2003-10-02) abstract	1-36
E	WO 03/096024 A (GOLZ STEFAN ; SUMMER HOLGER (DE); BAYER AG (DE); BRUEGGEMEIER ULF (DE)) 20 November 2003 (2003-11-20) abstract	1-36

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/10160

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 34,36 (partially)
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 34 and 36 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-36 (all partially)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 34 and 36 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Claims Nos.: 34,36 (partially)

Rule 39.1(iv) PCT - Diagnostic method practised on the human or animal body

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1- 36 (all partially)

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the FPRL1 polypeptide, a method to inhibit the expression of the FPRL1 and a method of diagnosis using mutations or expression levels of FPRL1 as indication.

---

2. claims: 1-36 (all partially)

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the FPRL2 polypeptide, a method to inhibit the expression of the FPRL2 and a method of diagnosis using mutations or expression levels of FPRL2 as indication.

---

3. claims: 1-36 (all partially)

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the FPR1 polypeptide, a method to inhibit the expression of the FPR1 and a method of diagnosis using mutations or expression levels of FPR1 as indication.

---

4. claims: 1-36 (all partially)

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the GPR32 polypeptide, a method to inhibit the expression of the GPR32 and a method of diagnosis using mutations or expression levels of GPR32 as indication.

---

5. claims: 1-36 (all partially)

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the CMKLR1 polypeptide, a method to inhibit the expression of the CMKLR1 and a method of diagnosis using mutations or expression levels of CMKLR1 as indication.

---

6. claims: 1-36 (all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the C5r1 polypeptide, a method to inhibit the expression of the C5R1 and a method of diagnosis using mutations or expression levels of C5R1 as indication.

---

7. claims: 1-36 (all partially)

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the GPR44 polypeptide, a method to inhibit the expression of the GPR44 and a method of diagnosis using mutations or expression levels of GPR44 as indication.

---

8. claims: 1-36 (all partially)

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the GCGR polypeptide, a method to inhibit the expression of the GCGR and a method of diagnosis using mutations or expression levels of GCGR as indication.

---

9. claims: 1-36 (all partially)

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the GLP1R polypeptide, a method to inhibit the expression of the GLP1R and a method of diagnosis using mutations or expression levels of GLP1R as indication.

---

10. claims: 1-36 (all partially)

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the GLP2R polypeptide, a method to inhibit the expression of the GLP2R and a method of diagnosis using mutations or expression levels of GLP2R as indication.

---

11. claims: 1-36 (all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the GIPR polypeptide, a method to inhibit the expression of the GIPR and a method of diagnosis using mutations or expression levels of GIPR as indication.

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12. claims: 1-36 (all partially)

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the VIPR1 polypeptide, a method to inhibit the expression of the VIPR1 and a method of diagnosis using mutations or expression levels of VIPR1 as indication.

---

13. claims: 1-36 (all partially)

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the SCTR polypeptide, a method to inhibit the expression of the SCTR and a method of diagnosis using mutations or expression levels of SCTR as indication.

---

14. claims: 1-36 (all partially)

A method to identify compounds influencing beta-amyloid precursor protein processing comprising checking the compound induced changes in activity of the VIPR2 polypeptide, a method to inhibit the expression of the VIPR2 and a method of diagnosis using mutations or expression levels of VIPR2 as indication.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/10160

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WO 03096024 A	20-11-2003	WO 03096024 A1	20-11-2003